Attorney Docket # 53000-111PUS Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Marvin A. SACKNER et al.

Serial No.: 10/566,872

Filed: February 2, 2006

For: Reciprocating Movement Platform for the External Addition of Pulses to the Fluid Channels of a Subject

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. MARVIN SACKNER

S I R:

Dr. Marvin Sackner hereby declares and states that:

1. I am an inventor of the above-identified application for a reciprocating movement platform for the external addition of pulses to the fluid channels of a subject.

2. This declaration presents evidence to counter the Examiner's rejection of claims 18-56 and 59-97 under 35 U.S.C. §101 because the claimed invention is not supported by either a specific and substantial utility or a well established utility.

3. Pages 13-40 of the original application (international application PCT/US2004/025017) provide various references to prior art related to the various treatments. The Examiner acknowledges that the prior art and specification provides reasonable support for the assertion that a reciprocating motion platform would serve to release nitric oxide within a patient undergoing treatment. The following demonstrate support for the assertion that the present invention provides effective treatments as recited in claims 18-56 and 59-97.

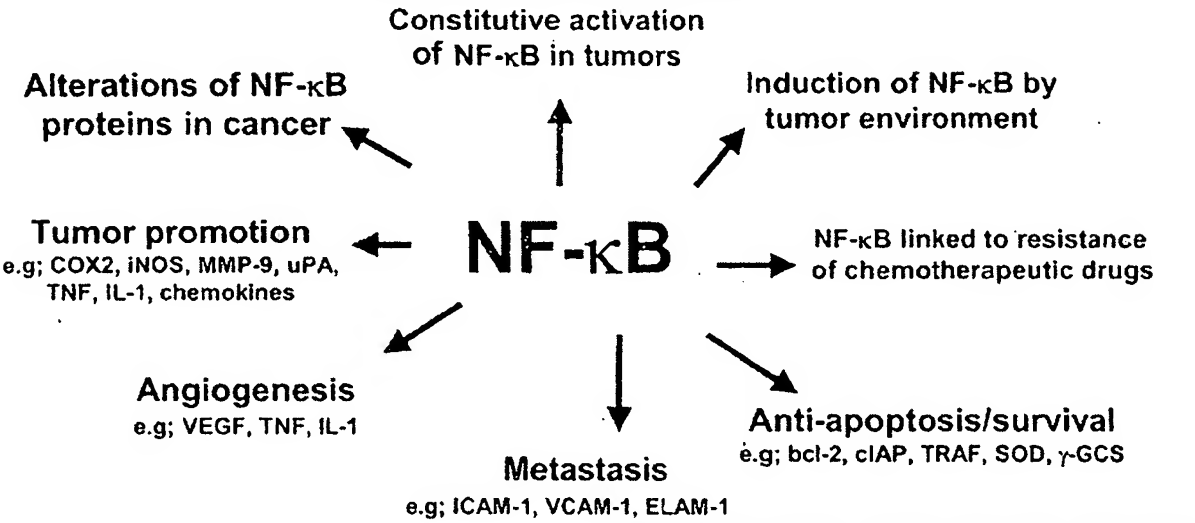
4. The present application is a Continuation-in-Part (CIP) of U.S. Patent Application 10/439,957, filed on May 15, 2003 (now U.S. Patent No. 7,111,346) (hereafter referred to as the Parent '957 Application).

Claim 18

5. Claim 18 recites "wherein the provided periodic acceleration is used to treat and/or to prevent cancers in tissues of the subject". This claim is based upon the suppression of nuclear factor kappa beta (NF-kB) by nitric oxide which is released from the vascular endothelium into the circulation of the subject through activation of endothelial nitric oxide synthase (eNOS). The Examiner has acknowledged that the reciprocating platform serves to release nitric oxide. As described below with respect to claim 19, the release of nitric oxide suppresses NF-kB.

6. Nitric oxide is released owing to increased pulsatile shear stress attendant with periodic acceleration. Further, the action of the motion platform produces pulsatile shear stress in all fluid channels of the body so that the release of nitric oxide is ubiquitous in the body and its inhibition of NF-kB is not limited by distribution issues. NF-kB has many actions that promote tumorogenesis (see figure below) and is the subject of many papers in the literature (see table below) on favorable effects on regression of tumors by blocking its activity. We demonstrated that application of periodic acceleration in an asthmatic sheep model was effective in blunting bronchospasm with antigen challenge and eliminating inflammation because of NF-kB inhibition (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752). Since NF-kB has been found to promote cancer progression, and the claimed periodic acceleration suppresses NF-kB, the use of periodic acceleration to treat or prevent cancer as recited in claim 18 has clear supporting evidence of its utility.

Mechanisms by which Nuclear Factor Kappa Beta (NF-kB) Promotes Cancer Progression



Garg A, Aggarwal BB. Nuclear transcription factor-kappaB as a target for cancer drug development. Leukemia 2002; 16:1053-1068.

# Papers* Dealing with Agents Inhibiting NF-κß that Diminish Experimental Tumors Alone or in Combination with Other Anti-Cancer Agents	
▪ Hodgkin's disease 20 ■ T cell lymphoma 7 ■ T cell leukemia 44 ■ Acute lymphoblastic leukemia 16 ■ Breast cancer 120 ■ Liver cancer 59 ■ Thyroid cancer 6 ■ Glioblastoma multiforma 17 ■ Kidney cancer 9 ■ Esophageal cancer 8	■ Pancreatic cancer 70 ■ Prostate cancer 108 ■ Melanoma 49 ■ Head & neck squamous cell cancer 14 ■ Colon cancer 97 ■ Multiple myeloma 53 ■ Ovarian cancer 29 ■ Bladder cancer 11 ■ Lung cancer 89 ■ Osteogenic sarcoma 16 ■ Kaposi's sarcoma 14
*National Medical Library Search July 2007	

Claim 19

7. Claim 19 recites "wherein the provided periodic acceleration causes release of nitric oxide from the vascular endothelium of the subject through activation of endothelial nitric oxide synthase (eNOS) which in turn suppresses nuclear factor kappa beta". Periodic acceleration causes release of nitric oxide from the vascular endothelium of the subject through activation of endothelial nitric oxide synthase (eNOS) that in turn suppresses the transcriptional factor, nuclear factor kappa beta, thereby suppressing formation of inflammatory cytokines, tumor necrosis factor, adhesion molecules, inducible nitric oxide synthase (iNOS) activity, and endothelin-1 release". It is well established in the scientific literature that activated nuclear factor kappa beta directs the suppression of inflammatory cytokines, tumor necrosis factor, adhesion molecules, inducible nitric oxide synthase (iNOS) activity, and endothelin-1 release, as depicted in the Table reproduced below.

TABLE 2. PROTEINS REGULATED BY NF-κB.

Proinflammatory cytokines
Tumor necrosis factor α
Interleukin-1β
Interleukin-2
Interleukin-6
Granulocyte–macrophage colony-stimulating factor
Macrophage colony-stimulating factor
Granulocyte colony-stimulating factor
Chemokines
Interleukin-8
Macrophage inflammatory protein 1α
Macrophage chemotactic protein 1
Gro-α, -β, and -γ
Eotaxin
Inflammatory enzymes
Inducible nitric oxide synthase
Inducible cyclooxygenase-2
5-Lipoxygenase
Cytosolic phospholipase A_2
Adhesion molecules
Intercellular adhesion molecule 1
Vascular-cell adhesion molecule 1
E-selectin
Receptors
Interleukin-2 receptor (α chain)
T-cell receptor (β chain)

Barnes, P. J. and Karin, M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med, 336: 1066-1071, 1997.

8. To evidence utility of claim 19, the following will demonstrate that the claimed invention produces nitric oxide at a sufficient level to suppress activated nuclear factor kappa beta, which suppresses inflammatory cytokines, tumor necrosis factor, adhesion molecules, inducible nitric oxide synthase (iNOS) activity, and endothelin-1 release. That the

claimed device causes release of nitric oxide from endothelial nitric oxide into the circulation through an increase of pulsatile shear stress to the endothelium has been demonstrated in an isolated aortic segment. Isolated porcine aortas exposed to nonpulsatile flow, pulsatile flow, and pulsatile flow plus periodic acceleration (pGz) produced with the present invention that added additional pulses exhibited an increase in nitrite measured with a nitric oxide electrode. The addition of pulsatile flow increased nitrites 300% relative to nonpulsatile flow which further increased to 1000%, relative to nonpulsatile flow (Adams JA, Moore JE, Jr., Moreno MR, Coelho J, Bassuk J, Wu D. Effects of periodic body acceleration on the in vivo vasoactive response to N-w-nitro-L-arginine and the in vitro nitric oxide production. Ann Biomed Eng 2003; 31:1337-1346).

9. Nitric oxide must be measured as nitrite, a metabolic marker of nitric oxide since this gas is metabolized within 5 seconds after release into the bloodstream. Serum nitrite was significantly elevated by the periodic acceleration in anesthetized pigs (Adams JA, Bassuk J, Wu D, Grana M, Kurlansky P, Sackner MA. Periodic acceleration: effects on vasoactive, fibrinolytic, and coagulation factors. J Appl Physiol 2005; 98(3):1083-1090). In rats exposed to periodic acceleration for 1 hour, the heart and lungs showed increased activity of the gene (eNOS) responsible for release of nitric oxide into the circulation (Wu H, Arias J, Bassuk J, Kurlansky P, Adams JA. Periodic acceleration (pGz) increases eNOS and nNOS in healthy rat heart. Circulation, abstract, in press, 2007).

10. In 10 volunteer subjects (5 with conditions that might respond to enhanced eNOS release and 5 controls). Periodic acceleration along the body's longitudinal axis was delivered through repetitive movements (2-3 Hz @ 0.25 G's) on a horizontal platform (AT101™ therapeutic table). Each subject underwent a single 45-minute session with brief interruptions to withdraw blood samples through an indwelling antecubital venous port at 0, 15, 30, and 45 minutes. Nanomolar concentrations of NO were measured that were well within the sensitivity of our assay and consistent with NO produced from eNOS. Periodic acceleration treatment increased plasma levels of NO in 7 out of 10 subjects. In 3 patients and 3 control subjects, the enhanced NO levels were significant either at 15, 30, or 45 minutes. In this study, mechanical stimulation using periodic acceleration elevated NO in the range associated with release from eNOS in the plasma of normal controls as well as individuals with disorders that have the potential to benefit from such elevations (Kuchera M.L., Daghigh F. Determination of enhanced nitric oxide production using external mechanical stimuli. JAOA 104: 344. abstract, 2004).

11. Serum nitrite is a metabolite of nitric oxide and is a marker that reflects NO release into the bloodstream but does not assure that the NO released has clinical or physiological effect. This point is discussed on pages 20 to 23 of the patent application. Nitric oxide as released into the bloodstream by the periodic acceleration according to the present invention causes descent of the dicrotic notch of the finger pulse. As mentioned in the patent application, this can be quantified by computing the a/b ratio. Comparison to known donor drugs of NO as listed on Table 1 following paragraph 0059 in the specification of the parent '957 application reveals that the peak response was significantly greater the NO donor drugs in terms of bioactivity. A larger group of subjects was reported with the same results (Sackner MA, Gummels E, Adams JA. Nitric oxide is released into circulation with whole-body, periodic acceleration. Chest 2005; 127:30-39).

12. The device of the claimed invention and cycling exercise that is also known to cause release of NO into the bloodstream were compared. It was found that dicrotic notch descent occurred during a brief bout of moderate cycling exercise, consistent with NO release into circulation. Periodic acceleration along the body's longitudinal axis (pGz) according to the recited invention produced comparable descent of the dicrotic notch but passive motorized cycling did not. In terms of the beneficial effects of NO, this study suggests that pGz according to the invention might serve as a substitute in subjects who are physically incapable of exercising (Sackner MA, Gummels E, Adams JA. Effect of moderate-intensity exercise, whole-body periodic acceleration, and passive cycling on nitric oxide release into circulation. Chest 2005; 128:2794-2803). The fall of dicrotic notch in the aorta of anesthetized rats also occurs with periodic acceleration and is greater than that seen after a NO donor drug (sodium nitroprusside) for an equivalent decrease in blood pressure. Further, the rise of systemic blood pressure after intravenous administration of L-NAME, an agent that blocks release of NO into the circulation is blunted with periodic acceleration (Uryash A, Wu H, Bassuk J, Kurlansky P, Sackner MA, Adams JA. Nitroprusside, L-NAME and whole body, periodic acceleration in rats. Circulation , abstract, in press. 2007; Uryash A, Wu H, Bassuk J, Kurlansky P, Adams JA, Sackner MA. Whole body periodic acceleration blunts hypertensive action of L-NAME in rats. Circulation, abstract, in press. 2007.

13. The following examples will show that NO released into the bloodstream with action of the claimed invention has a role in the treatment of inflammatory diseases now also known as "nuclear factor kappa beta diseases" that includes diseases cited in claim 19 (Appendix A attached to this declaration includes a list of such inflammatory diseases). Here it is necessary to demonstrate beneficial effects as well as suppression of nuclear factor kappa beta (NF-kB) activity.

14. To measure NF-kB activity, it is necessary to obtain cells from the inflammatory site – it cannot be measured in plasma. Using the allergic (asthmatic) sheep model as described in paragraphs 0061 to 0064 of the parent '957 specification, nuclear factor kappa beta was measured 6 hours after antigen challenge at the time of the late airways response (LAR). These studies revealed that the periodic acceleration according to the present invention induces release of NO from eNOS that protects against acute allergen-induced responses, most likely by modulating mast cell activity (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752). More prolonged treatment prevents airways hyperreactivity. To determine if pGz according to the invention suppresses NF-kB activity thereby affecting antigen-induced airway responses, bronchoalveolar lavage 6h after antigen challenge was carried out and free p65 levels in lavage cell nuclear extracts (an indicator of NF-kB activation) was determined with ELISA. Peak LAR (% increase over baseline) in control, pGz-treated and L-NAME+pGz treated sheep (all n=6) were 118+2%, 21+4% and 130+4%, respectively. Levels of p65/106 cells were 1.9- and 1.8-fold higher in the control and L-NAME+pGz groups (both $p<0.05$) when compared to pGz treated animals. Therefore, pGz according to the invention stimulates eNOS and increases NO throughout the body, which can block NF-kB–mediated inflammation.

15. For other nuclear factor kappa beta diseases, SF-36v2 quality of life questionnaires were obtained after ten to fifteen, 45-minute treatments in five patients with chronic fatigue syndrome and/or fibromyalgia. Significant improvements were observed in three of the eight categories, e.g., Role Physical, Body Pain, and Vitality (Sackner MA, Adams JA. Periodic acceleration causing release of nitric oxide improves health related quality of life in chronic inflammatory diseases. International Symposium on Nitric Oxide, Cytokines and Inflammation, 2004).

16. **Basis for Utility of Claim 19.** The above paragraphs show that the release of nitric oxide suppresses nuclear factor kappa beta.

Claim 20

17. Claim 20 recites "wherein the provided periodic acceleration serves as a means for preconditioning, conditioning and/or postconditioning tissues of the body of the subject". Evidence to support the utility of claim 20 is found at paragraphs 0067-0077 of the specification and relates to the fact that whole body periodic acceleration increases pulsatile shear stress thereby activating endothelial nitric oxide synthase to release NO. More recent references are summarized to support this claim follow below.

18. *Preconditioning* consists of performing a beneficial intervention prior to the ischemic event to minimize ischemic damage during reperfusion, *conditioning* a beneficial intervention during the ischemic event and prior to reperfusion, and *postconditioning* a beneficial intervention after reperfusion. With reperfusion of the ischemic organs, tissues may undergo damage with resultant cellular death. This occurs as follows: reperfusion of ischemic tissues is associated with microvascular dysfunction that is manifested as impaired endothelium-dependent dilation in arterioles, enhanced fluid filtration and leukocyte plugging in capillaries, and the trafficking of leukocytes and plasma protein extravasation in postcapillary venules. Activated endothelial cells in all segments of the microcirculation produce more free oxygen radicals, but less nitric oxide, in the initial period following reperfusion. The resulting imbalance between superoxide and nitric oxide in endothelial cells leads to the production and release of inflammatory mediators (e.g. platelet-activating factor, tumor necrosis factor alpha) and enhances the biosynthesis of adhesion molecules that mediate leukocyte-endothelial cell adhesion. Some of the known risk factors for cardiovascular disease (hypercholesterolemia, hypertension, and diabetes) appear to exaggerate many of the microvascular alterations elicited by ischemia and reperfusion (I/R). The inflammatory mediators released as a consequence of reperfusion also appear to activate endothelial cells in remote organs that are not exposed to the initial ischemic insult. This distant response to I/R can result in leukocyte-dependent microvascular injury that is characteristic of the multiple organ dysfunction syndrome (Carden, D. L. and Granger, D. N. Pathophysiology of ischaemia-reperfusion injury. J Pathol, *190*: 255-266, 2000).

19. Ischemic *preconditioning* (IPC) was first identified and defined by the classical paper of Murry et al in 1986 (Murry, C.E., Jennings, R.B. and Reimer, KE. Preconditioning with ischemia: a delay of lethal injury in ischemic myocardium, Circulation 1986;74:1124–1136) which demonstrated that four 5 min periods of ischemia prior to a

prolonged 40 min of occlusion and 72 h of reperfusion resulted in a marked decrease in myocardial infarct size compared to control hearts which did not receive the preconditioning stimulus. Subsequently, two windows of cardioprotection were identified, an early period in which the protective effect only lasted 1–3 h and a later or delayed phase in which the protection lasts from 24 to 72 h. The early phase carries much more significance than the later phase. This phenomenon was shown to occur in all species tested including man and the reduction in infarct size was remarkably similar across species. Several endogenously present pharmacological agents were shown to mimic the cardioprotective effects of IPC including adenosine, bradykinin, opioids, acetylcholine, erythropoeitin (EPO) and nitric oxide. Note that bradykinin activates eNOS. Also pretreatment with drugs that enhance NO release such as statins, certain calcium antagonists, ACE inhibitors, and corticosteroids produce preconditioning and protect the heart against ischemia. (Schulz, R., Kelm, M., and Heusch, G. Nitric oxide in myocardial ischemia/reperfusion injury. Cardiovasc Res, *61*: 402-413, 2004). Preconditioning with endothelin receptor antagonists preserves endothelial and cardiac contractile function by a mechanism that is dependent upon endothelial nitric oxide production (Gonon, A. T., Erbas, D., Broijersen, A., Valen, G., and Pernow, J. Nitric oxide mediates protective effect of endothelin receptor antagonism during myocardial ischemia and reperfusion. Am J Physiol Heart Circ Physiol, *286*: H1767-H1774, 2004).

20. Preconditioning also minimizes impaired ventricular contraction, propensity to arrhythmias and myocardial stunning that may occur with reperfusion. The impaired ventricular contraction is due to hypercontracture of the myocardium due to increased calcium influx into cardiomyocytes. In cardiac surgery when hearts are reperfused after prolonged ischemia, reperfusion may provoke "stone heart," i.e., a stiff and pale heart characterized by massive muscle contracture and loss of cellular protein content (Piper, H. M., Abdallah, Y., and Schafer, C. The first minutes of reperfusion: a window of opportunity for cardioprotection. Cardiovasc Res, *61*: 365-371, 2004). Genetic overexpression of endothelial nitric oxide in mice attenuates myocardial infarct size after ischemia/reperfusion (Jones, S. P., Greer, J. J., Kakkar, A. K., Ware, P. D., Turnage, R. H., Hicks, M., van, H. R., de, C. R., Kawashima, S., Yokoyama, M., and Lefer, D. J. Endothelial nitric oxide synthase overexpression attenuates myocardial reperfusion injury. Am J Physiol Heart Circ Physiol, *286*: H276-H282, 2004).

21. Most investigators agree that the basis for the early phase of ischemic preconditioning relates to activation of endothelial nitric oxide synthase (eNOS). The basis for the late phase of cardiac protection from preconditioning (PC) has been controversial. Bolli and associates up until 2007 have championed the importance of iNOS in this process (Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. J Mol Cell Cardiol 2001; 33(11):1897-1918). They have presented evidence that enhanced biosynthesis of NO by eNOS as essential to trigger the late phase of ischemia-induced and exercise induced PC, and enhanced NO production by iNOS is obligatorily required to mediate the anti-stunning and anti-infarct actions of late PC elicited by five different stimuli (ischemia, adenosine A1 agonists, opioid agonists, endotoxin derivatives and exercise). Therefore, they hypothesized that NO plays a dual role in the pathophysiology of the late phase of PC, acting initially as the trigger and subsequently as the mediator of this adaptive response. The diversity of the PC stimuli that

converge on iNOS implies that the upregulation of this enzyme is a central mechanism whereby the myocardium protects itself from ischemia. The NO hypothesis of late PC thus implies a cytoprotective function of iNOS in the heart, a novel paradigm which has recently been extended to other tissues, including kidney and intestine. Other corollaries of this hypothesis are that the heart responds to stress in a biphasic manner, utilizing eNOS as an immediate but short-term response and iNOS as a delayed but long-term defense, and that the fundamental difference between non-preconditioned and late preconditioned myocardium is the tissue level of iNOS-derived NO, which is tonically higher in the latter compared with the former. Hence, late PC can be viewed as a state of enhanced NO synthesis.

22. However, Laude et al presented evidence that activation of eNOS rather than iNOS during reperfusion is a mediator of late phase cardiac preonditioning (Laude, K., Favre, J., Thuillez, C., and Richard, V. NO produced by endothelial NO synthase is a mediator of delayed preconditioning-induced endothelial protection. Am J Physiol Heart Circ Physiol, *284*: H2053-H2060, 2003). Recently, Bolli with new genetic techniques disproved his previous findings indicating that activation of iNOS was responsible for the late phase of PC and confirmed that eNOS was the trigger for both the early and late phases (Xuan YT, Guo Y, Zhu Y, Wang OL, Rokosh G, Bolli R. Endothelial nitric oxide synthase plays an obligatory role in the late phase of ischemic preconditioning by activating the protein kinase C{epsilon}-p44/42 mitogen-activated protein kinase-pSer-signal transducers and activators of transcription1/3 pathway. Circulation 2007, in press). In mice bred such that the eNOS gene is absent develop adverse vascular remodeling (intimal proliferation, decreased lumen size and flow) and increased necrosis after ischemia-reperfusion as well as increased expression of P-selectin. Absence of eNOS has an adverse effect in ischemia reperfusion injury. Finally, nitric oxide from eNOS inhibits activity of Angiotensin II that is upregulated in ischemia reperfusion injury (Jugdutt, B. I. Nitric oxide and cardioprotection during ischemia-reperfusion. Heart Fail Rev, *7*: 391-405, 2002).

23. The later protection phase of preconditioning appears to be the result of suppression of an inflammatory response. During reperfusion, nuclear factor kappa beta is activated along with expression of several NF-κß regulated genes. These include leukocyte adhesion molecules, cytokines, tumor necrosis alpha, and chemokines (Valen, G., Yan, Z. Q., and Hansson, G. K. Nuclear factor kappa-B and the heart. J Am Coll Cardiol, *38*: 307-314, 2001). Certain pharmacological agents also inhibit nuclear translocation of NF-κß from the inactive form in the cytoplasm. The experimental drug (IMD-0354) not only reduces harmful neutrophil accumulation in the myocardium but also inhibits inflammatory cytokine and chemokine production by cardiomyocytes (Onai, Y., Suzuki, J., Kakuta, T., Maejima, Y., Haraguchi, G., Fukasawa, H., Muto, S., Itai, A., and Isobe, M. Inhibition of IkappaB phosphorylation in cardiomyocytes attenuates myocardial ischemia/reperfusion injury. Cardiovasc Res, *63*: 51-59, 2004).

24. In terms of preconditioning of the heart, we conclude that the above information is ample to support our claim 20 that whole body periodic acceleration, through the application of a motion platform, effectively achieves preconditioning. This was confirmed in a recent study of 20 anesthetized male swine weighing 40-50lb (Adams JA, Bassuk J, Wu H, Arias J, Wu D, Jarapur V et al. Preconditioning with periodic acceleration (pGz) prior to whole body

ischemia reperfusion injury ameliorates myocardial stunning and arrhythmias. Circulation, abstract, in press. 2007). The pigs were placed on a motion platform and randomized to 1 hr of active pGz (3 Hz and Gz ± 0.4) (PC) or no activation for the same time period, control(C). Ventricular fibrillation (VF) was electrically induced and unsupported for 8 min, followed by continuous manual chest compression and defibrillation until return of spontaneous circulation (ROSC) or a maximum period of 10 min. Echocardiograms to measure ejection fraction (EF%), fractional shortening (FS%) and wall motion score index (WMSI) were performed at baseline (BL), after pGz or control (BL2) and 30, 120 min after ROSC (ROSC30, ROSC120). All animals had ROSC after a median of 4 defibrillation attempts. PC animals had less hemodynamically significant arrhythmias in the first 30 mins ROSC; C (35) vs PRE (7) ($p< 0.05$) and less myocardial stunning as determined by echocardiography. Operation of the periodic acceleration according to the present invention causes release of NO from eNOS through increased pulsatile shear stress. Further, preconditioning with the periodic acceleration according to the present invention is non-invasive and drug-free. It is now clear from numerous peer-reviewed publications that various preconditioning methods or drugs have their basis in the early and later phases in activated eNOS. Furthermore, particularly in the late phase, an inflammatory response mediated by activation of NF-κß plays an important role in myocardial dysfunction. As we have shown for a sheep model of allergic asthma, the periodic acceleration according to the present invention activates NO from eNOS thereby blunting bronchoconstriction and suppressing NF-κß activity in bronchoalveolar lavage fluid (see Reference 20 (Appendix A)).

25. The above-described mechanisms are operative for eNOS activation in the early and later phases of preconditioning as a direct effect of NO release and indirectly in other organs through suppression of nuclear factor kappa beta activity as listed below.

Preconditioning to protect brain

- Ischemic preconditioning to protect the brain against more severe ischemic insults has its basis in activation of both endothelial and neuronal nitric oxide synthases with activation of the former a more consistent finding (Atochin, D. N., Clark, J., Demchenko, I. T., Moskowitz, M. A., and Huang, P. L. Rapid cerebral ischemic preconditioning in mice deficient in endothelial and neuronal nitric oxide synthases. Stroke, *34*: 1299-1303, 2003; Centeno, J. M., Orti, M., Salom, J. B., Sick, T. J., and Perez-Pinzon, M. A. Nitric oxide is involved in anoxic preconditioning neuroprotection in rat hippocampal slices. Brain Res, *836*: 62-69, 1999; Gidday, J. M., Shah, A. R., Maceren, R. G., Wang, Q., Pelligrino, D. A., Holtzman, D. M., and Park, T. S. Nitric oxide mediates cerebral ischemic tolerance in a neonatal rat model of hypoxic preconditioning. J Cereb Blood Flow Metab, *19*: 331-340, 1999; Hashiguchi, A., Yano, S., Morioka, M., Hamada, J., Ushio, Y., Takeuchi, Y., and Fukunaga, K. Up-regulation of endothelial nitric oxide synthase via phosphatidylinositol 3-kinase pathway contributes to ischemic tolerance in the CA1 subfield of gerbil hippocampus. J Cereb Blood Flow Metab, *24*: 271-279, 2004). The above references indicate that the brain can be preconditioned by activating endothelial nitric oxide synthase through increased pulsatile shear stress. Therefore, these references support utility of claim 33 that the periodic acceleration according to the present invention preconditions tissues of the body.

Preconditioning to protect kidney

- Ischemic preconditioning to protect the kidney against more severe ischemic insults that could result in renal failure has its basis in activation of endothelial nitric oxide synthase (Yamasowa, H., Shimizu, S., Inoue, T., Takaoka, M., and Matsumura, Y. Endothelial nitric oxide contributes to the renal protective effects of ischemic preconditioning. J Pharmacol Exp Ther, *312*: 153-159, 2005). The increase of NO released from eNOS suppresses endothelin-1, a potent vasoconstricting agent that is increased in acute renal failure (Kurata, H., Takaoka, M., Kubo, Y., Katayama, T., Tsutsui, H., Takayama, J., Ohkita, M., and Matsumura, Y. Protective effect of nitric oxide on ischemia/reperfusion-induced renal injury and endothelin-1 overproduction. Eur J Pharmacol, *517*: 232-239, 2005). These references indicate that the kidneys can be preconditioned by activating endothelial nitric oxide synthase through increased pulsatile shear stress. Therefore, these references support utility of claim 33 that the periodic acceleration according to the present invention preconditions tissues of the body.

Preconditioning to protect lungs

- Hypoxic preconditioning protects the lungs against the deleterious effects of severe hypoxia as might occur in high altitude. Survival in severe hypoxia such as occurs in high altitude requires previous acclimatization, which is acquired over a period of days to weeks. Mice pretreated with whole-body hypoxic preconditioning (WHPC, 6 cycles of 10-min hypoxia-10-min normoxia) survived significantly longer than control animals when lethal hypoxia was administered (5% O2, survival time of 33 min vs. controls at 14 min, $n = 10$, $p < 0.005$). This protective mechanism becomes operative shortly after WHPC and remains effective for at least 8 h. Mice subjected to WHPC demonstrated improved gas exchange when exposed to sublethal hypoxia (7% O2, arterial blood PO2 of 50 vs. controls at 40 Torr, $n = 6$, $p < 0.05$), reduced formation of pulmonary edema (increase in lung water of 0.49 vs. controls at 0.89 mg/mg dry tissue, $n = 10$, $p < 0.02$), and decreased pulmonary vascular permeability (lung lavage albumin of 7.6 vs. controls at 18 mg/dl, $n = 6$-10, $p < 0.025$). The severity of cerebral edema caused by exposure to sublethal hypoxia was also reduced after WHPC (increase in brain water of 0.25 vs. controls at 0.49 mg/mg dry tissue, $n = 10$, ($p < 0.01$). Thus, WHPC protects unacclimatized mice against acute and otherwise lethal hypoxia, and this protection involves preservation of vital organ functions (Zhang, S. X., Miller, J. J., Gozal, D., and Wang, Y. Whole-body hypoxic preconditioning protects mice against acute hypoxia by improving lung function. J Appl Physiol, *96*: 392-397, 2004).

- High-altitude pulmonary edema (HAPE) is a potentially fatal condition, which usually occurs at altitudes in excess of 3000 m and affects fit and previously well individuals. It appears that a genetic basis renders susceptibility to this hypoxic insult. Data have been presented that susceptible individuals (HAPE-S) may have deficiency of NO, a molecule that dilates the pulmonary vasculature. Thus, preconditioning with the periodic acceleration according to the present invention by upregulating eNOS activity should prevent the development of HAPE (Duplain, H., Sartori, C., Lepori, M., Egli, M., Allemann, Y., Nicod, P., and Scherrer, U. Exhaled nitric oxide in high-altitude pulmonary edema: role in the regulation of pulmonary vascular tone and evidence for a role against inflammation. Am J Respir Crit Care

Med, *162* : 221-224, 2000; Mortimer, H., Patel, S., and Peacock, A. J. The genetic basis of high-altitude pulmonary oedema. Pharmacol Ther, *101*: 183-192, 2004).

- Since hypoxic preconditioning activates endothelial nitric oxide synthase (Muzaffar, S., Shukla, N., Angelini, G. D., and Jeremy, J. Y. Acute hypoxia simultaneously induces the expression of gp91phox and endothelial nitric oxide synthase in the porcine pulmonary artery. Thorax, *60*: 305-313, 2005; Yamamoto, Y., Henrich, M., Snipes, R. L., and Kummer, W. Altered production of nitric oxide and reactive oxygen species in rat nodose ganglion neurons during acute hypoxia. Brain Res, *961*: 1-9, 2003), it is likely that the NO released from activated eNOS protects the lungs during subsequent severe hypoxia, as reflected by improved arterial oxygenation, reduced formation of pulmonary edema, and decreased pulmonary vascular permeability. This is borne out by investigations related to improved arterial oxygenation with inhaled nitric oxide or intravenous sodium nitroprusside (Mestan, K. K., Carlson, A. D., White, M., Powers, J. A., Morgan, S., Meadow, W., and Schreiber, M. D. Cardiopulmonary effects of nebulized sodium nitroprusside in term infants with hypoxic respiratory failure. J Pediatr, *143*: 640-643, 2003; Mourani, P. M., Ivy, D. D., Gao, D., and Abman, S. H. Pulmonary vascular effects of inhaled nitric oxide and oxygen tension in bronchopulmonary dysplasia. Am J Respir Crit Care Med, *170*: 1006-1013, 2004). Overexpression of eNOS reduces pulmonary edema and deficiency of eNOS promotes high altitude pulmonary edema (Droma, Y., Hanaoka, M., Ota, M., Katsuyama, Y., Koizumi, T., Fujimoto, K., Kobayashi, T., and Kubo, K. Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. Circulation, *106*: 826-830, 2002; Jones, S. P., Greer, J. J., van, H. R., Duncker, D. J., de, C. R., and Lefer, D. J. Endothelial nitric oxide synthase overexpression attenuates congestive heart failure in mice. Proc Natl Acad Sci U S A, *100*: 4891-4896, 2003). Endothelial nitric oxide synthase maintains the low basal permeability of the vascular endothelium thereby preventing extravasation of albumin into the extravascular spaces (Predescu, D., Predescu, S., Shimizu, J., Miyawaki-Shimizu, K., and Malik, A. B. Constitutive eNOS-derived nitric oxide is a determinant of endothelial junctional integrity. Am J Physiol Lung Cell Mol Physiol, *289*: L371-L381, 2005). eNOS attenuates post-ischemic inflammatory injury to the lung probably via inhibition of adhesion molecules (Kaminski, A., Pohl, C. B., Sponholz, C., Ma, N., Stamm, C., Vollmar, B., and Steinhoff, G. Up-regulation of endothelial nitric oxide synthase inhibits pulmonary leukocyte migration following lung ischemia-reperfusion in mice. Am J Pathol, *164*: 2241-2249, 2004). The above references evidence the utility of using the claimed invention for preconditioning the lungs of a body and thus evidence of the utility of preconditioning tissues of the body to events that are associated with at least one of impaired blood supply and delivery of oxygen to the tissues.

26. Additional references support that preconditioning with the periodic acceleration according to the present invention to upregulate eNOS will be effective in suppressing pulmonary ischemia reperfusion injury.

- Ischemic preconditioning of the stomach protects against mucosal damage induced by ischemia reperfusion injury and topical mucosal irritants to the stomach. This protection is lost with administration of L-NAME, a NOS inhibitor. This indicates that nitric oxide has a key role in gastric ischemic preconditioning (Konturek, S. J., Brzozowski, T., Pajdo, R., Konturek, P. C., Kwiecien, S., Sliwowski, Z., Pawlik, M., Ptak, A., Drozdowicz, D., and

Hahn, E. G. Gastric preconditioning induced by short ischemia: the role of prostaglandins, nitric oxide and adenosine. Med Sci Monit, *7*: 610-621, 2001). Ischemic preconditioning protects the intestines against mucosal damage induced ischemia reperfusion injury (Mallick, I. H., Yang, W., Winslet, M. C., and Seifalian, A. M. Ischemia-reperfusion injury of the intestine and protective strategies against injury. Dig Dis Sci, *49*: 1359-1377, 2004; Mallick, I. H., Yang, W., Winslet, M. C., and Seifalian, A. M. Ischaemic preconditioning improves microvascular perfusion and oxygenation following reperfusion injury of the intestine. Br J Surg, *92*: 1169-1176, 2005). These references indicate that nitric oxide has a key role in gastric ischemic preconditioning. Since the present invention activates endothelial nitric oxide synthase through increased pulsatile shear stress, these references support utility of using the claimed invention to precondition tissues of the body.

- Ischemic preconditioning of the liver protects against ischemia reperfusion injury to the liver and remotely to the pancreas. Its basis resides in activation of eNOS (Koti, R. S., Tsui, J., Lobos, E., Yang, W., Seifalian, A. M., and Davidson, B. R. Nitric oxide synthase distribution and expression with ischemic preconditioning of the rat liver. FASEB J, *19*: 1155-1157, 2005; Dembinski, A., Warzecha, Z., Ceranowicz, P., Tomaszewska, R., Dembinski, M., Pabianczyk, M., Stachura, J., and Konturek, S. J. Ischemic preconditioning reduces the severity of ischemia/reperfusion-induced pancreatitis. Eur J Pharmacol, *473*: 207-216, 2003; Peralta, C., Closa, D., Hotter, G., Gelpi, E., Prats, N., and Rosello-Catafau, J. Liver ischemic preconditioning is mediated by the inhibitory action of nitric oxide on endothelin. Biochem Biophys Res Commun, *229*: 264-270, 1996; Peralta, C., Fernandez, L., Panes, J., Prats, N., Sans, M., Pique, J. M., Gelpi, E., and Rosello-Catafau, J. Preconditioning protects against systemic disorders associated with hepatic ischemia-reperfusion through blockade of tumor necrosis factor-induced P-selectin up-regulation in the rat. Hepatology, *33*: 100-113, 2001). These references indicate that activation of eNOS preconditions the liver and pancreas. Since the periodic acceleration according to the present invention activates endothelial nitric oxide synthase through increased pulsatile shear stress, these references evidence utility of the recitation that the periodic acceleration according to the present invention preconditions tissues of the body.

- Ischemic preconditioning of skeletal muscle protects against ischemia reperfusion injury to the skeletal muscle (Kuntscher, M. V., Kastell, T., Altmann, J., Menke, H., Gebhard, M. M., and Germann, G. Acute remote ischemic preconditioning II: the role of nitric oxide. Microsurgery, *22*: 227-231, 2002). This reference indicates that nitric oxide plays a role in preconditioning of skeletal muscle. Since the periodic acceleration according to the present invention activates endothelial nitric oxide synthase through increased pulsatile shear stress, these references evidence utility of the recitation that the periodic acceleration according to the present invention preconditions tissues of the body.

27. Regarding "conditioning the tissues", this is a new concept that originates from our research with the periodic acceleration according to the present invention. To better understand this concept, "postconditioning" must first be defined. This intervention consists of producing brief episodes of ischemia during the first few minutes of reperfusion. Conditioning occurs during the sustained ischemic episode and prior to reperfusion. It cannot be readily accomplished with pharmacologic agents owing to impaired blood flow nor is it possible to attain such an effect with an invasive procedure. But conditioning is attainable through

operation of the periodic acceleration according to the present invention during the ischemic phase since pulses can be added to the circulation even during low perfusion states. To demonstrate effectiveness of conditioning, it must be shown that regional blood flows to tissues are markedly reduced during the ischemic episode but that function of such tissues returns toward pre-ischemic conditions following reperfusion. To demonstrate conditioning, a porcine model of ventricular fibrillation model supported solely by the action of the claimed invention was used.

- The application of the claimed invention at 2 Hz increased cardiac output in juvenile pigs in ventricular fibrillation proportional to the amplitude of the applied acceleration force that plateaued at 0.7 G. Cardiac output in fibrillating animals was restored to 20% of the values obtained prior to fibrillation with the periodic acceleration according to the present invention. In another study, capillary blood flow was determined before and after pGz-CPR using colored microspheres. Capillary perfusion was detected in all tissue beds studied during pGz-CPR. Significant capillary blood flow was detected in the endocardium and brain stem during pGz-CPR that represented 39 and 197% of control values before fibrillation, respectively. In a final group, animals were successfully resuscitated with return of spontaneous circulation (ROSC) after pGz-CPR for 15 min following ventricular fibrillation with a 3-min non-intervention period. Following ROSC, blood pressure was maintained at pre-arrest values for 2 h without any pharmacological or mechanical support. None of the control animals (18 min of fibrillation without pGz-CPR) survived the experimental protocol and only two of these six animals briefly returned to spontaneous circulation (<20 min) (Adams JA, Mangino MJ, Bassuk J, Kurlansky P, Sackner MA. Novel CPR with periodic Gz acceleration. Resuscitation 2001; 51:55-62). The preceding study demonstrates that acute ischemia reperfusion injury is minimized through "conditioning" during induced global ischemia (ventricular fibrillation).

- The ultimate assessment of the value of any CPR technique is the neurological outcome after using such a technique. Ventricular fibrillation was induced in 12 juvenile pigs. After a 3 min non-interventional interval, the animals received either the periodic acceleration according to the present invention (n=7) or no intervention (n=5) for 15 min. After 18 min of ventricular fibrillation, defibrillation was attempted. All animals in the periodic acceleration group had return of spontaneous circulation (ROSC) and normal neurological assessment at 24 h. Neurological outcome remained normal at 48 h. In contrast, none of the animals in the no intervention group had ROSC (Adams JA, Bassuk J, Wu D, Kurlansky P. Survival and normal neurological outcome after CPR with periodic Gz acceleration and vasopressin. Resuscitation 2003; 56:215-221). Therefore, prolonged application of the periodic acceleration according to the present invention in global ischemia minimized or eliminated clinical evidence of neurological reperfusion injury.

- Application of the periodic acceleration according to the present invention in older pigs with induced ventricular fibrillation produced similar rates of Return Of Spontaneous Circulation (ROSC) as reported by other investigators in pigs without the risk of rib fractures. Further, this method was associated with a lower incidence of periods of hemodynamic instability following ROSC than conventional closed chest massage with a "thumper" device (Adams JA, Wu D, Bassuk J, Kurlansky P. Cardiopulmonary resuscitation (CPR) using periodic

acceleration (pGz) in an older porcine model of ventricular fibrillation. Resuscitation 2004; 60:327-334).

- The effects of conventional cardiopulmonary resuscitation (CPR) using an automated Thumper™ chest compression device was compared to the periodic acceleration according to the present invention on early post-resuscitation ventricular function in an adult porcine model of CPR as assessed by echocardiography. Ventricular fibrillation was induced in 16 animals (25-35kg). After 3min of non-interventional period, the animals were randomized to receive either the periodic acceleration according to the present invention or the Thumper for 15min. After 18min of ventricular fibrillation, defibrillation was attempted. An echocardiogram was performed at baseline and serially for 6h. Return Of Spontaneous Circulation (ROSC) to 360min occurred in 5/8 (62%) of the animals receiving Thumper and in 7/8 (88%) receiving the periodic acceleration according to the present invention. Fractional shortening of the left ventricle and ejection fraction were impaired after CPR, but the animals subjected to periodic acceleration according to the present invention had significantly less impairment than those in the Thumper group. Further, wall motion score index was significantly more impaired after Thumper than the present invention group and remained as such even 6h post-CPR. This study suggests that the periodic acceleration according to the present invention conditioned the heart during the global ischemic period of ventricular fibrillation (Nava G, Adams JA, Bassuk J, Wu D, Kurlansky P, Lamas GA. Echocardiographic comparison of cardiopulmonary resuscitation (CPR) using periodic acceleration (pGz) versus chest compression. Resuscitation 2005; 66:91-97).

- Regional blood flow studies in adult pigs with induced ventricular fibrillation with a 3 minute non-intervention period followed by 15 minute application of the periodic acceleration according to the present invention or Thumper™ (mechanical closed chest compression device for CPR) were done with colored microspheres (Adams et al. unpublished study). Myocardial and endocardial blood flows declined to <5% of their pre-ventricular fibrillation values during application of the present invention and to ~20% of the pre-ventricular fibrillation value with the Thumper. However, the postresuscitation cardiac function as measured with echocardiography after the present invention application was superior to the Thumper as measured in a different group of pigs as in the Reference 16 above. In another study, pretreatment with the eNOS inhibitor, L-NAME gave much poorer outcome with the present invention than the Thumper indicating that nitric oxide released during the periodic acceleration according to the present invention is essential to conditioning.

- The aforementioned studies show that "conditioning" of the myocardium takes place with application of the present invention during the global ischemia of ventricular fibrillation and prior to reperfusion. Conditioning is mediated by release of nitric oxide from eNOS because of increased pulsatile shear stress produced with the periodic acceleration according to the present invention. Therefore, there is sufficient evidence to support claim 33 that periodic acceleration according to the claimed invention provides "conditioning" to the heart and brain and presumable other tissues since the animals in described above had normal neurological function 48 hours after the ischemic episode and also appeared healthy as indicated

by their daily activity, eating, urinary and bowel functioning (Adams JA, Bassuk J, Wu D, Kurlansky P. Survival and normal neurological outcome after CPR with periodic Gz acceleration and vasopressin. Resuscitation 2003; 56:215-221).

28. Brief intermittent episodes of ischemia and reperfusion, at the onset of reperfusion after a prolonged period of ischemia, confer cardioprotection, a phenomenon termed "ischemic *postconditioning*" (Tsang, A., Hausenloy, D. J., Mocanu, M. M., and Yellon, D. M. Postconditioning: a form of "modified reperfusion" protects the myocardium by activating the phosphatidylinositol 3-kinase-Akt pathway. Circ Res, *95*: 230-232, 2004). Tsang used isolated perfused rat hearts that were subjected to: (a) 35 minutes of ischemia and 120 minutes of reperfusion, determined infarct size by tetrazolium staining; or (b) 35 minutes of ischemia and reperfusion followed by six 10 second cycles of reperfusion followed by 10 minutes reperfusion. Postconditioning reduced infarct size from 51% to 32%, an effect comparable to ischemic preconditioning (IPC) and was accompanied by a significant increase in eNOS activity. Staat, P., Rioufol, G., Piot, C., Cottin, Y., Cung, T. T., L'Huillier, I., Aupetit, J. F., Bonnefoy, E., Finet, G., ndre-Fouet, X., and Ovize, M (Postconditioning the human heart. Circulation, *112*: 2143-2148, 2005) performed postconditioning in 30 patients undergoing coronary angioplasty for acute myocardial infarction. After reperfusion by direct stenting, control subjects underwent no further intervention but postconditioning was performed within 1 minute of reflow by 4 episodes of 1-minute inflation and 1-minute deflation of the angioplasty balloon. Infarct size was assessed by measuring total creatine kinase release over 72 hours. Total creatine kinase reflects myocardial damage. The area under the curve of creatine kinase release over time was significantly reduced by postconditioning. compared with the control group equating to a 36% reduction in infarct size. Thus, postconditioning by coronary angioplasty protects the human heart during acute myocardial infarction.

29. Endothelial nitric oxide synthase with NO release is a mediator of postconditioning. Postconditioning is blocked by prior administration of L-NAME, an inhibitor of eNOS (Tsang, A., Hausenloy, D. J., and Yellon, D. M. Myocardial postconditioning: reperfusion injury revisited. Am J Physiol Heart Circ Physiol, *289*: H2-H7, 2005). Further, it appears that postconditioning is as effective as preconditioning in reducing myocardial infarct size and preserving endothelial function (Zhao, Z. Q., Corvera, J. S., Halkos, M. E., Kerendi, F., Wang, N. P., Guyton, R. A., and Vinten-Johansen, J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol, *285*: H579-H588, 2003). As pointed out by Zhao and associates, postconditioning may be clinically applicable in coronary interventions, coronary arterial bypass surgery, organ transplantation, and peripheral revascularization where reperfusion injury is expressed. Adams and associates (Adams JA, Bassuk J, Wu H, Arias J, Wu D, Jarapur V et al. Post treatment with periodic acceleration (pGz) after cardiac arrest decreases acute post resuscitation myocardial stunning. Circulation, abstract, in press, 2007) demonstrated that periodic acceleration produced post conditioning. Eighteen anesthetized male swine (40-50lbs) were studied. Ventricular fibrillation (VF) was electrically induced and unsupported for 8 min, followed by continuous manual chest compression and defibrillation until return of spontaneous circulation (ROSC) or 10 min. They were randomized to receive continuous pGz (frequency of 3 Hz and Gz ± 0.4) (POST CONDITIONING) for the remainder of the observation period or none, control (C). Echocardiograms to measure ejection fraction, fractional shortening and wall motion

score index were performed at baseline (BL), and 30, 120 min after ROSC (ROSC30, ROSC120). All animals had ROSC after a median of 4 defibrillation attempts. There were no differences between groups in defibrillation attempts, time to ROSC, arterial blood gases or hemodynamics over time. Compared to Controls, POST CONDITIONED animals had less acute myocardial stunning as evidenced by echocardiographic indices.

30. **Basis for Utility of Claim 20.** The above references indicate that postconditioning is mediated by activation of endothelial nitric oxide synthase. This evidences utility of using the claimed invention to activates eNOS by increased pulsatile shear stress and thereby non-invasively produce postconditioning as recited in claim 20.

Claim 21

31. Claim 21 recites "wherein treatment with periodic acceleration before, during, or after athletic performance prevents and/or treats tissue damage, reduces systemic stress, increases athletic performance, and/or prevents/treats any of the problems caused by strenuous athletic activity".

32. Skeletal Muscle Damage. It has long been recognized that strenuous exercise as might occur during athletic performance is associated with skeletal muscle damage. Thus, biopsies of the gastrocnemius muscles of volunteer human marathon runners were extracted prior to and at intervals for 7 days following a marathon, and investigated ultrastructurally. Most of the preparations, including the pre-marathon samples, showed evidence of muscle fiber necrosis and inflammation. These abnormal conditions were most prevalent at 1 and 3 days after the marathon. These ultrastructural changes are compared and correlated with the reports of clinical manifestations of rhabdomyolysis and myoglobinuria. Moderate intensity exercise of a very prolonged interval (e.g., 33 hours in ultramarathon) can induce asymptomatic exertional rhabdomyolysis. Because the abnormalities persist for the 7 day duration of these observations, and because many of these were observed in the pre-marathon biopsies, both the intensive training for, and the marathon itself, induce inflammation and fiber necrosis which are manifested in the clinical symptoms of delayed onset of muscle soreness [DOMS] (Hikida RS, Staron RS, Hagerman FC, Sherman WM, Costill DL. Muscle fiber necrosis associated with human marathon runners. J Neurol Sci 1983; 59(2):185-203; (Skenderi KP, Kavouras SA, Anastasiou CA, Yiannakouris N, Matalas AL. Exertional Rhabdomyolysis during a 246-km continuous running race. Med Sci Sports Exerc 2006; 38:1054-1057).

33. Tissue Damage due to Oxidative Stress, Ischemia/Reperfusion Injury, Inflammation. The damage to tissues from strenuous exercise is related to oxidative stress, ischemia/reperfusion injury and inflammation. A major contributor to oxidative stress associated with exercise is the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that produce ischemia/reperfusion injury. During exhaustive or anaerobic exercise, blood flow is diverted to skeletal muscles (although not necessarily in sufficient quantity) and other tissues, such as the liver, may become ischemic. Upon cessation of exercise, both these tissues deprived of oxygenated blood are flooded with ROS and RNS that damage tissues. The figure below depicts the potential effects of exercise as it relates to increase of ROS and RNS on muscular fatigue. Also, NF-κ is activated by oxidative stress. It should be noted that oxidative

stress does not occur with moderate exercise intensity (<50% maximum oxygen consumption) since the body's natural antioxidant capacity is not overwhelmed. However, more strenuous exercise produces oxidative stress (Finaud J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. Sports Med 2006; 36:327-358; Aoi W, Naito Y, Takanami Y, Kawai Y, Sakuma K, Ichikawa H et al. Oxidative stress and delayed-onset muscle damage after exercise. Free Radic Biol Med 2004; 37:480-487).

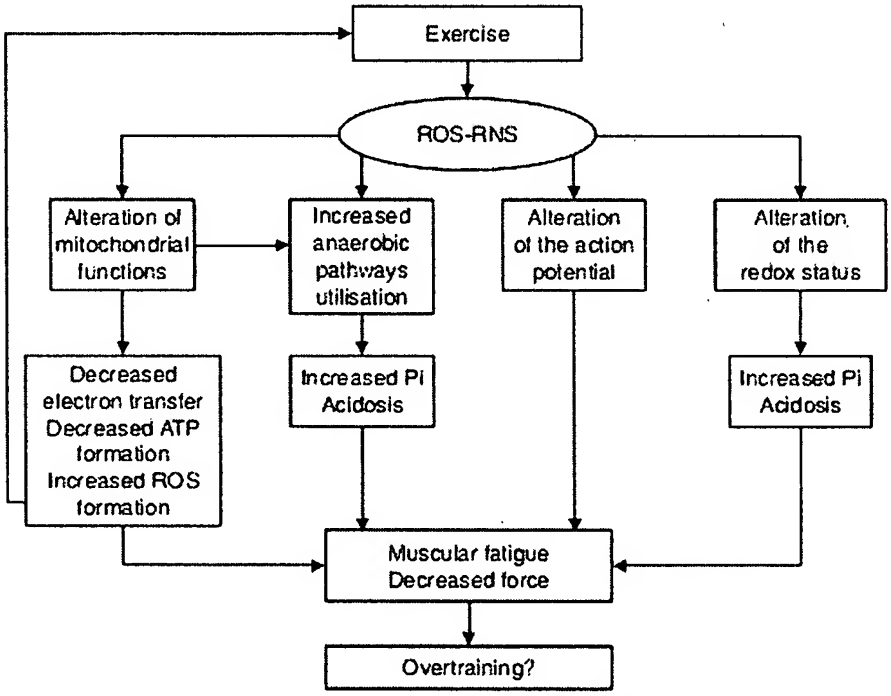


Fig. 3. The different hypothesis about the effects of reactive oxygen species (ROS) on muscular fatigue. **ATP** = adenosine triphosphate; **redox** = oxidation-reduction; **RNS** = reactive nitrogen species; **Pi** = inorganic phosphate.

34. Antioxidants and Reactive Oxygen and Nitrogen Species. An antioxidant is a substance that helps to reduce the severity of oxidative stress either by forming a less active radical or by quenching the damaging ROS/RNS chain reaction on substrates such as proteins, lipids, carbohydrates or DNA. Aerobic, anaerobic or mixed training provokes a decrease of oxidative stress, which is caused by an increase of the efficiency of the antioxidant system in response to the supplementary production of ROS and RNS during exercise. The training program must be sufficiently long and intense to trigger a consequent adaptive response of the antioxidant system and a decrease of oxidative stress. This adaptation is more important when the training level of the subjects is low at the beginning of the protocol. This training-induced improvement of the antioxidant status and decrease of oxidative stress are extensively documented in the literature. However, there may be a decrease of antioxidant system efficiency, particularly in high-level athletes subjected to an important training and competitive load with an inappropriate diet. These studies suggest a limit beyond which oxidative stress can increase in excess and cause overtraining. The level of ROS and RNS produced during exercise play an important role not only in the induction of muscular lesions but also in the induction and propagation of post-exercise inflammation, which can increase cellular lesions. These

phenomena can disrupt muscular functions and lead to the overtraining syndrome. (Finaud J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. Sports Med 2006; 36:327-358). Nitric oxide released from eNOS as a result of increased pulsatile shear stress through treatment with the motion platform scavenges reactive oxygen and nitrogen species, i.e., ROS and RNS (Espey MG, Miranda KM, Thomas DD, Xavier S, Citrin D, Vitek MP et al. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. Ann N Y Acad Sci 2002; 962:195-206).

35. Inflammation and Neutrophil Endothelial Interaction. Strenuous exercise can produce inflammation in muscles that lead to muscle damage. If inflammation is regarded as the proliferation of white blood cells after soft tissue injury, then the cellular inflammatory response actually begins at the onset of exercise, when the circulating level of neutrophils significantly increases. Neutrophils arrive in muscle and affect the host inflammatory response during exercise and soft tissue injury. These cells have both specific and nonspecific defensive mechanisms, some of which are capable of causing additional tissue damage. The mechanism for early neutrophilia in the postexercise state is likely due to a combination of factors. During rest, more than half of the circulating neutrophils are marginated along the endothelial walls of blood vessels. At the onset of exercise, increases in epinephrine, blood flow, and cell-signaling molecules demarginate these neutrophils away from the vessel walls, resulting in their mobilization into the circulation. Demargination allows the neutrophils to enter the circulation and redistribute elsewhere in the body, as needed.

36. Recruitment of Neutrophils and Mast Cells. The movement of a neutrophil from the circulation into the tissue, called diapedesis, is under tight regulatory control of the underlying tissue. In skeletal muscle, diapedesis can occur rapidly during exercise. Neutrophil recruitment is ultimately the responsibility of the muscle fibers (myocytes) together with mast cells from a variety of tissues, including the local connective tissue. If a myocyte is perturbed in some fashion, such as in the case of an active stretch or contusion, it communicates with the endothelial wall of the adjacent blood vessel, initiating a cascade of signaling events and resulting in diapedesis. This intercellular communication is accomplished, in part, by a series of cell-signaling molecules, or cytokines.

37. Cytokines. All nucleated cells in the body produce cytokines and similarly express cytokine receptors on their surface membranes. Cytokines act at the surface of the target cells, principally to alter cell function. Skeletal muscle continually produces cytokines in an effort to maintain homeostasis and to regulate function. Simple perturbations of skeletal muscle, such as an active stretch during eccentric exercise, markedly increase the expression of interleukin-1ß, (IL-1ß) and tumor necrosis factor–α (TNF-α). These proinflammatory cytokines upregulate the expression of endothelial leukocyte adhesion molecules (E-selectin) within the endothelium of the adjacent blood vessels. Activation of the endothelium can result in the release of additional IL-1ß, as well as additional proinflammatory cytokines, including IL-6 and IL-8, both of which have been shown to attract neutrophils. Thus, endothelial activation serves 2 purposes: encouraging the adhesion of neutrophils at the site of cell stress (margination) and assisting the cell in recruiting additional neutrophils (Figure 1). (Butterfield TA, Best TM, Merrick MA. The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair. J Athl Train 2006; 41:457-465).

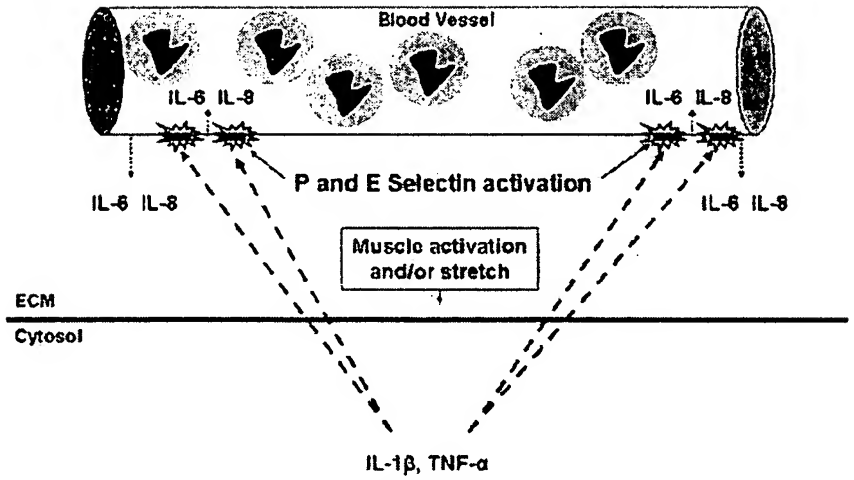


Figure 1. Modified muscle use may result in an increased intracellular calcium concentration, resulting in an increased cell production of proinflammatory cytokines such as TNF-α and IL-1β, which in turn upregulate the expression of endothelial-leukocyte adhesion molecules (E-selectin and P-selectin). The activated endothelium attracts neutrophils to the region and also releases the neutrophil chemoattractants and proinflammatory cytokines IL-6 and IL-8. IL-6 indicates interleukin 6; IL-8, interleukin 8; IL-1β, interleukin 1-beta; TNF-α, tumor necrosis factor–alpha; 1β; TNF-α, tumor necrosis factor–α; and ECM, extracellular matrix.

38. Membrane Disruption, Neutrophils, Cytokines. Membrane disruption is probably the initial, propagating event in muscle injury owing to the high forces transmitted during eccentric exercise. In this manner, membrane disruption causes neutrophils to migrate to the damaged area in order to remove the damaged tissue through phagocytosis. Exacerbation of injury can take place after eccentric exercise, including observations that the initial injury is often followed by a secondary loss of muscle force, due to additional but delayed damage to the muscle fibers. This so-called secondary damage has been proposed to be caused by invading neutrophils, potentially due to a second burst of neutrophilia within 24 hours of cessation of exercise. More importantly, this secondary burst appears to mediate damage through the release of cytotoxic compounds. This secondary response likely results from bone marrow release of neutrophils in response to elevated blood catecholamine levels. These neutrophils appear to be more oxidatively active than the first group of neutrophils emigrating to the extracellular matrix (ECM). After muscle injury, myocytes and other cells release cytokines, such as IL-1ß, IL-6, IL-8, and TNF-α, that activate neutrophils to produce a host of cytotoxic substances, including ROS, such as superoxide anions, hypochloride, and hydrogen peroxide. The cytokines IL-1ß, IL-6, and TNF-α all stimulate pathways that contribute to activation of the enzyme NADPH oxidase in neutrophils and endothelium, which generates a ''respiratory burst'' and the subsequent release of reactive oxygen species.

39. Ischemia/Reperfusion Injury. Ischemia/reperfusion injury demonstrates the damaging actions of neutrophils in the absence of prior tissue injury. When blood flow is restored to ischemic muscle, neutrophils quickly marginate and rapidly extravasate into the muscle ECM and myocytes. Although tissue reperfusion clearly provides a significant stress to the tissues (i.e., metabolic stress), notable in these situations is the absence of mechanical tissue injury or trauma. The removal of neutrophils from experimental animal models of ischemia-reperfusion results in less oxidant production and the attenuation of membrane disruption during the reperfusion of ischemic tissue. The essential step in the inflammatory process is the adhesion of neutrophils to the endothelium, which ultimately leads to neutrophil-mediated cell destruction and necrosis. The primary mechanism of tissue destruction is the release of cytotoxic agents by the invading neutrophils, in the absence of any initial mechanical event.

40. Treatment of Musculoskeletal Injuries. Historically, the acute management of athletic musculoskeletal injury has focused on limiting the cardinal signs of inflammation in an attempt to expedite the rehabilitative process and to facilitate an early return to competition. To this end, the use of ice, compression, and elevation for initial management of injuries has flourished. Over the last 25 years, rationales for acute treatment practices have changed, focusing on retarding secondary injury in an effort to minimize total injury. Regardless of the rationale, the practice of using ice, compression, and elevation in managing acute inflammation is well ingrained but not evidenced based. Similarly, limiting inflammation and enhancing tissue repair through the suppression of neutrophil recruitment and activation may reduce tissue damage postexercise. Such efforts relate to administration of nonsteroidal inflammatory drugs (NSAIDs).

41. Anti-Inflammatory Pathways and Treatment. The anti-inflammatory effects of NSAIDs may be confounded by the analgesic action of these drugs, which has long been the focus of early interventions for muscle injury. It has been suggested that the magnitude of pain after tissue trauma corresponds to the concentration of WBCs within the injured tissue. But this approach has not been supported by scientific studies. For example, although tendinitis is a common diagnosis, the absence of WBCs in tissues affected by this condition indicates that this is not a true inflammatory response. Conversely, the mere presence of WBCs does not always coincide with the cardinal signs of inflammation. White blood cells have been observed in the absence of obvious tissue trauma, even though this situation is generally not referred to as an inflammatory process. A challenge to reducing inflammation through pharmacologic intervention is the multiple cellular pathways by which the inflammatory response can be mediated. Traditional NSAIDs block the cyclooxygenase (COX) pathway that contributes to cell-mediated prostaglandin (PGE2) production and neutrophil recruitment. However, other proinflammatory pathways exist for the cell to recruit neutrophils to damaged or exercised muscle, including the alternative lipoxygenase pathway and nuclear factor kappa-beta (NF-κß)–mediated induction of proinflammatory genes. For instance, inhibition of NF-κß by curcumin accelerates muscle regeneration whereas non–NF-κß inhibitors such as the NSAID naproxen do not affect regeneration (Butterfield TA, Best TM, Merrick MA. The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair. J Athl Train 2006; 41:457-465).

42. Heat Stroke. This is a special example of muscle damage that occurs with strenuous exercise and is characterized as a systemic condition that includes core body temperature (Tc) >40°C, a systemic inflammatory response (SIR), central nervous system dysfunction (coma, delirium, convulsion), disseminated intravascular coagulation (DIC) and multi-organ failure (MOF). Hot, dry skin and cardiovascular deterioration may be observed at times. Increased Tc associated with strenuous exercise is not believed to be the basis for these symptoms although it may serve as a trigger for the pathophysiology. Rather, endotoxemia seems to be the most important factor for heat stroke based upon observations that heat stroke patients share a similar clinical presentation as patients with sepsis and that the methods to treat sepsis increase heat tolerance and survival in animals experiencing heat stroke. With heat stress, the permeability of the gut epithelium may be compromised by cutaneous vasodilation and splanchnic vasoconstriction, due to the effects of high temperature, oxidant injury, ischaemia/reperfusion, and recruitment of inflammatory cells. Increased gut permeability allows LPS residing in the gut flora to migrate into the portal circulation. This process overwhelms the liver with LPS, which leaks into the central circulation (endotoxemia) and induces SIR, resulting in necrosis, DIC, MOF, and other symptoms commonly observed in heat stroke patients.

43. Myocardial Ischemia. Strenuous, prolonged exercise as in running a marathon may produce abnormal left ventricular function as determined by echocardiography and biochemical markers of myocardial injury. These findings probably due to ischemia, a deficiency in oxygenated blood flow relative to demands of the cardiac muscle, are generally mild but can last as long as 24 hours after the initial insult. But athletes that need to perform on a daily basis such as Tour de France bicycle riders or basketball players, such damage might limit aerobic exercise performance (Middleton N, Shave R, George K, Whyte G, Forster J, Oxborough D et al. Novel application of flow propagation velocity and ischaemia-modified albumin in analysis of postexercise cardiac function in man. Exp Physiol 2006; 91:511-519; Shave R, Dawson E, Whyte G, George K, Gaze D, Collinson P. Altered cardiac function and minimal cardiac damage during prolonged exercise. Med Sci Sports Exerc 2004; 36:1098-1103; Tulloh L, Robinson D, Patel A, Ware A, Prendergast C, Sullivan D et al. Raised troponin T and echocardiographic abnormalities after prolonged strenuous exercise--the Australian Ironman Triathlon. Br J Sports Med 2006; 40:605-609). Myocardial ischemia may be ameliorated by preconditioning, postconditioning or chronic preconditioning through nitric oxide from activation of eNOS (Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. J Mol Cell Cardiol 2001; 33:1897-1918; Wang Y, Ahmad N, Wang B, Ashraf M. Chronic preconditioning: a novel approach for cardiac protection. Am J Physiol Heart Circ Physiol 2007; 292(5):H2300-H2305). All the conditioning protocols can be accomplished with motion platform treatments.

44. Infections in Elite Athletes. Upper respiratory illness (URI) is the most common medical condition affecting elite athletes. Thirty-two elite and 31 recreationally competitive triathletes and cyclists, and 20 sedentary controls (age range 18.0-34.1 yr) participated in a prospective surveillance study. Nasopharyngeal and throat swabs were collected from subjects presenting with two or more defined upper respiratory symptoms. Thirty-seven URI episodes were reported in 28 subjects. Incidence rate ratios for illness were higher in both the control subjects (1.93, 95% CI: 0.72-5.18) and elite athletes (4.50, 1.91-10.59)

than in the recreationally competitive athletes. Infectious agents were identified in only 11 (two control, three recreationally competitive, and six elite) out of 37 illness episodes. Rhinovirus was the most common respiratory pathogen isolated. Symptom and functional impairment severity scores were higher in subjects with an infectious pathogen episode, particularly on illness days 3-4. These results confirm a higher rate of URI among elite athletes than recreationally competitive athletes during this training and competition season (Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG et al. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 2007; 39:577-586). The incidence of symptoms of upper respiratory tract illness is increased in the days following prolonged strenuous endurance events and it has been generally assumed that this is due to the temporary exercise-induced depression of immune function. More recently it has been proposed that at least some of these symptoms are attributable to inflammation of the upper respiratory tract rather than to infectious episodes (Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG et al. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 2007; 39:577-586). In either case suppression of nuclear factor kappa beta that is elevated in tissues with strenuous exercise (Bar-Shai M, Carmeli E, Reznick AZ. The role of NF-kappaB in protein breakdown in immobilization, aging, and exercise: from basic processes to promotion of health. Ann N Y Acad Sci 2005; 1057:431-447) by NO released from eNOS through the motion platform should minimize episodes of infection or inflammation. This will enable the athlete to perform at his/her highest level.

45. Gastrointestinal Mucosal Integrity and Gut Blood Flow. The GI mucosa separates the body's sterile internal environment from the non-sterile environment of the gut. A plethora of >400 species of bacteria live in the gut space; LPS is located in the walls of gram-negative bacteria. These bacteria are harmless when confined to the gut, but can be harmful when they migrate across the gut epithelial tissue into the circulation. Although a small amount of bacteria routinely leaks through the gut epithelium, the bacteria are efficiently detoxified and cleared by the liver without affecting the central circulatory environment. Despite its critical role as a 'gatekeeper' of the sterile internal environment, the gut does not respond well to prolonged exercise. Blood flow to the intestine is reduced by about 80% during exercise and this reduction is exacerbated by hyperthermia. Reduced blood flow to the gut may persist for some time after prolonged exercise. For example, splanchnic blood flow was reduced by 60% for 90 minutes after a 20km run. Splanchnic partial pressure of oxygen (pO2) and pH declined significantly and hypoxia-labelled cells in the intestine increased significantly during hyperthermia. Gut ischemia and hypoxia increases the permeability of the paracellular tight junctions in the gut epithelium. As a consequence, larger molecules in the gut space that would not normally pass through the epithelial tight junction at rest can be translocated across the gut membrane into the portal circulation under exposure to exercise and heat stress.

46. Clinical Gastrointestinal Disturbances in Strenuous Exercise. Alterations in membrane permeability and ischemia in the gut accompany GI disturbances in long-distance runners. Symptoms of GI disturbances in endurance runners include abdominal cramps, diarrhea, vomiting, nausea and bloody stool. A survey of 134 runners reported that diarrhea (47%), bloody stool (16%), and severe lower abdominal cramps (36%) occurred frequently after intense running. It has been reported that 13–42% of marathon runners experienced GI bleeding in six different marathons. Others have observed GI bleeding in 8–22% of marathon runners and

85% of ultra-marathon runners. Along with these GI symptoms, endotoxemia has also been documented at the end of ultra endurance events. Marathon runners who developed bloody stools were significantly younger and ran faster times than those with normal stools. An abrupt increase in training volume is also associated with GI bleeding. High-intensity running at 80% maximum oxygen consumption (VO2max) significantly induces greater gut epithelial. Bloody diarrhea in runners has been attributed to hyperthermia-induced gut ischemia. This is consistent with the observation that rats with Tc of 42.5°C had higher gut permeability than those with lower Tc and with the histological analysis of gut tissues that revealed intestinal epithelial damage in rats heated to Tc >41°C. Examination of marathon runners 95–180 minutes after the race using GI endoscopy and a creatinine-labelled macromolecule biomarker revealed weaknesses of mucosal resistance, reflecting damage to the intercellular junctions, increased intestinal permeability, gastric mucosal erosion, and bleeding in the abdomen and small intestine. Thus, GI disturbances caused by long-distance running can promote the translocation of LPS from the gut into the circulation (figure 1). This observation is consistent with the occurrence of endotoxemia and heat stroke among long-distance runners and triathletes (Lim CL, Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. Sports Med 2006; 36:39-640).

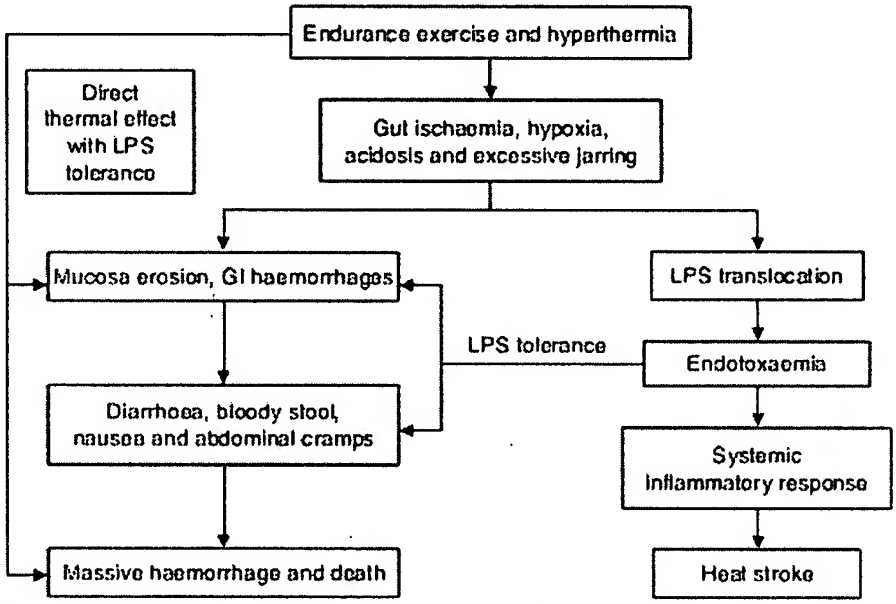


Fig. 1. Gastrointestinal (GI) response during endurance exercise and heat stress. Endurance exercise and hyperthermia cause erosion of the mucosa in the GI tract, and promote lipopolysaccharide (LPS) translocation from the gut into the portal circulation. Excessive LPS translocation can result in endotoxaemia, which eventually leads to heat stroke. Athletes who are tolerant to LPS continue to exercise intensely, which may cause other forms of GI disturbances (e.g. diarrhoea, bloody stool, nausea, abdominal cramps and haemorrhages) that can be fatal in extreme cases.

47. Single Bout of Stressful Exercise. The immune response to a single bout of exercise has been investigated in exercises such as running (1–3 hours), rowing (2 hours), interval swimming, cycling and weight training. Most studies have documented leukocytosis (+53% to +261%), monocytosis (+18% to +256%), granulocytosis (+32% to +396%), neutrophilia (+78% to +260%), and lymphopenia (–19% to –60%), which persist for ≥1 hour after exercise. The lymphopenia is accompanied by reduced concentrations of natural killer

(NK) cells (–60% to –80%), B cells (–8% to 40%), T cells (–14% to –50%), T-suppressor cells (-40% to –60%), and T-helper cells (–5% to –52%). These immune changes are transient and resting values are restored within 6–24 hours. Two immune functions affected by prolonged exercise are NK cell cytotoxic activity (NKCA) and the proliferative response of lymphocytes when challenged with a mitogen in vitro. NKCA decreases by 43% to 61% for up to 2 hours after strenuous exercise. NK cells may be shifted from the circulation into other lymphoid compartments through the effects of cortisol or may enter damaged skeletal muscles as part of the tissue repair process. Lymphocyte proliferation in response to phytohaemagglutinin and Cancavalin A stimulation declines after strenuous exercise. The decline in lymphocyte function is due to the disproportionate increase in the ratio of NK to T cells in the circulation. NK cells mediate non-major histocompatablity complex (MHC)-restricted cytotoxicity and offer potential resistance to viral infections. NK cells are involved in scavenging LPS from the blood and T cells activate B cells, which are essential for anti-LPS antibody production. Acute suppression of NKCA and the lymphocyte proliferative response after intense exercise may reflect compromised anti-LPS activities. If so, athletes performing prolonged intense exercise may have reduced capability to scavenge and neutralise LPS translocated during exercise and heat stress, which will increase the risk of endotoxemia-driven heat stroke and reduce heat tolerance.

48. Compromised Immune System with Intense Exercise. Prolonged intense exercise increases the risk of upper respiratory tract infection (URTI). For example, in the 2 months before the Los Angeles Marathon, 43.2% of runners sampled (n = 2311) reported at least one bout of URTI; 12.9% of those who were well before the race experienced symptoms of URTI in the week after the race. Two other studies reported that 28–33% of runners had symptoms of URTI in the 2 weeks following a 56km race; these rates were significantly higher than in non-athlete control subjects (2.9–15.3%) who lived in the same household as runners but did not participate in the race. Other researchers have directly observed long-term adaptation of the immune system to exercise by imposing an exercise regime over a period of time. For example, 4 weeks of anaerobic training and 8–15 weeks of moderate intensity aerobic training did not significantly change immune cell concentrations and functions in untrained, middle-aged to elderly subjects. In trained runners, no significant changes in the capacity of neutrophils to produce reactive oxygen species were observed after 800km of running during a 1-month training camp. In contrast, elite swimmers undergoing 7 months but not 12 weeks of intensive training exhibited a 57% reduction in NK cell concentration and 35% reduction in NK cell proportion of lymphocyte cells. Long-term aerobic training enhanced NKCA and lymphocyte proliferative responses in the elderly, compared with age-matched counterparts who had undergone 12 weeks of aerobic training. These results suggest that short-term (4-week) high-intensity and medium-term (8–15 weeks) moderate-intensity training do not significantly alter the immune system. High-intensity training for >6 months may reduce NK cell concentration. However, exercise habits that are sustainable over the long term (years) appear to enhance NK and lymphocyte cell functions. The figures 1-3 shown below depict the changes of the immune system and the risk of infections following exercise of different intensities (Pedersen BK, Rohde T, Ostrowski K. Recovery of the immune system after exercise. Acta Physiol Scand 1998; 162:325-332).

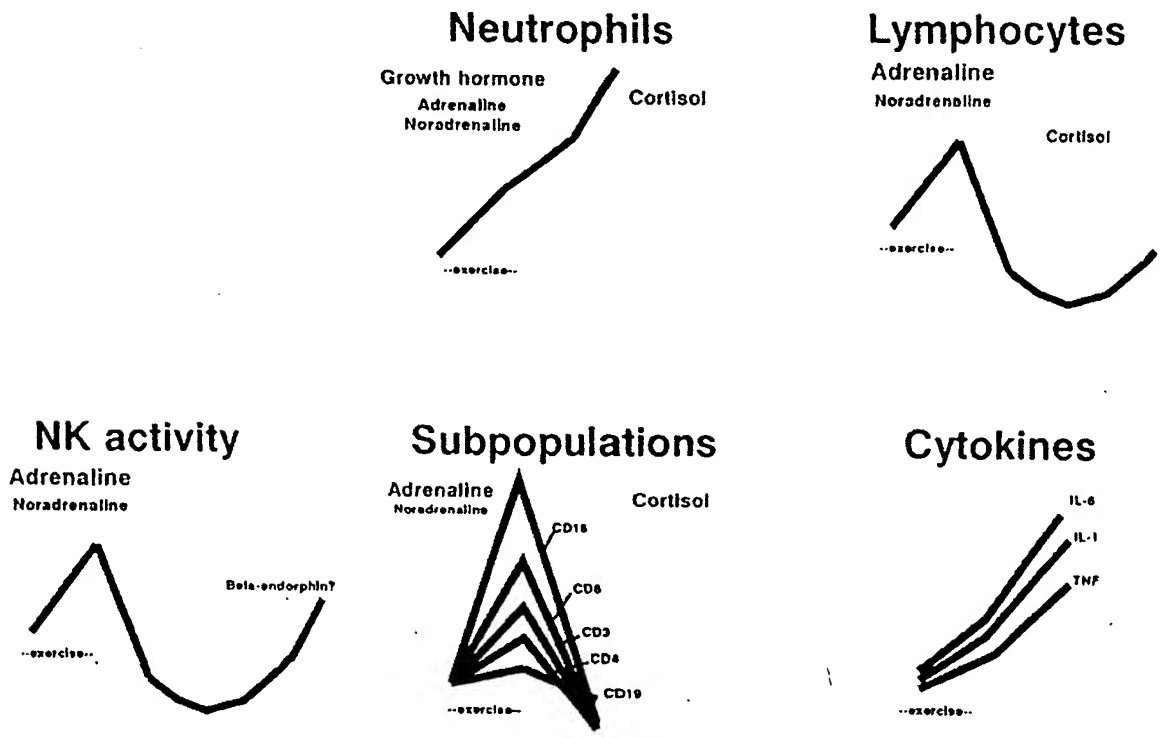


Figure 1 Model of the possible roles of stress hormones in mediating exercise-related immunological changes. Adrenaline and, to a lesser degree, noradrenaline are responsible for the acute effects of exercise on lymphocyte count and NK cell activity. Catecholamines and growth hormone in combination are responsible for the effects on neutrocytes. Cortisol contributes to maintain lymphopenia and neutrocytosis only after long term stress.

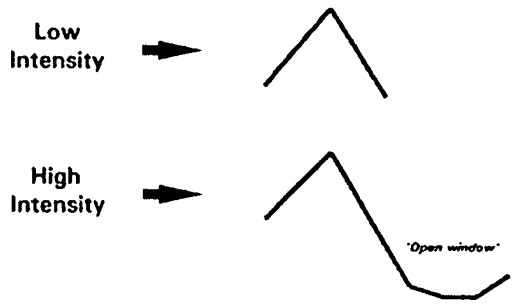


Figure 2 Schematic presentation of the effects of exercise at low and high intensity with fixed duration of time. In the model is included the exercise-effects on lymphocyte number, natural killer and lymphokine activated killer cell activities and antibody production. In contrast to moderate exercise, intense exercise is followed by a period of immunosuppression during which there is an 'open window' of opportunity for pathogens.

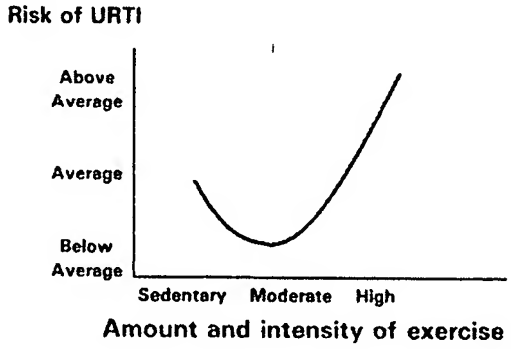


Figure 3 'J'-shaped model of the relationship between varying amounts of exercise and risk of upper respiratory tract infection (URTI). This model suggests that moderate exercise may lower the risk of respiratory infection while excessive amounts may increase risk.

Pedersen et al Recovery of the immune system after exercise. Acta Physiol Scand 1998;162:325-332

49. Cytokines are increased during heat stroke. Whereas heat-induced endotoxemia triggers the onset of heat stroke, the systemic inflammatory response activated by the increasing level of circulating LPS drives the clinical progression to heat stroke (figure 2). (Lim CL, Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. Sports Med 2006; 36:39-640).

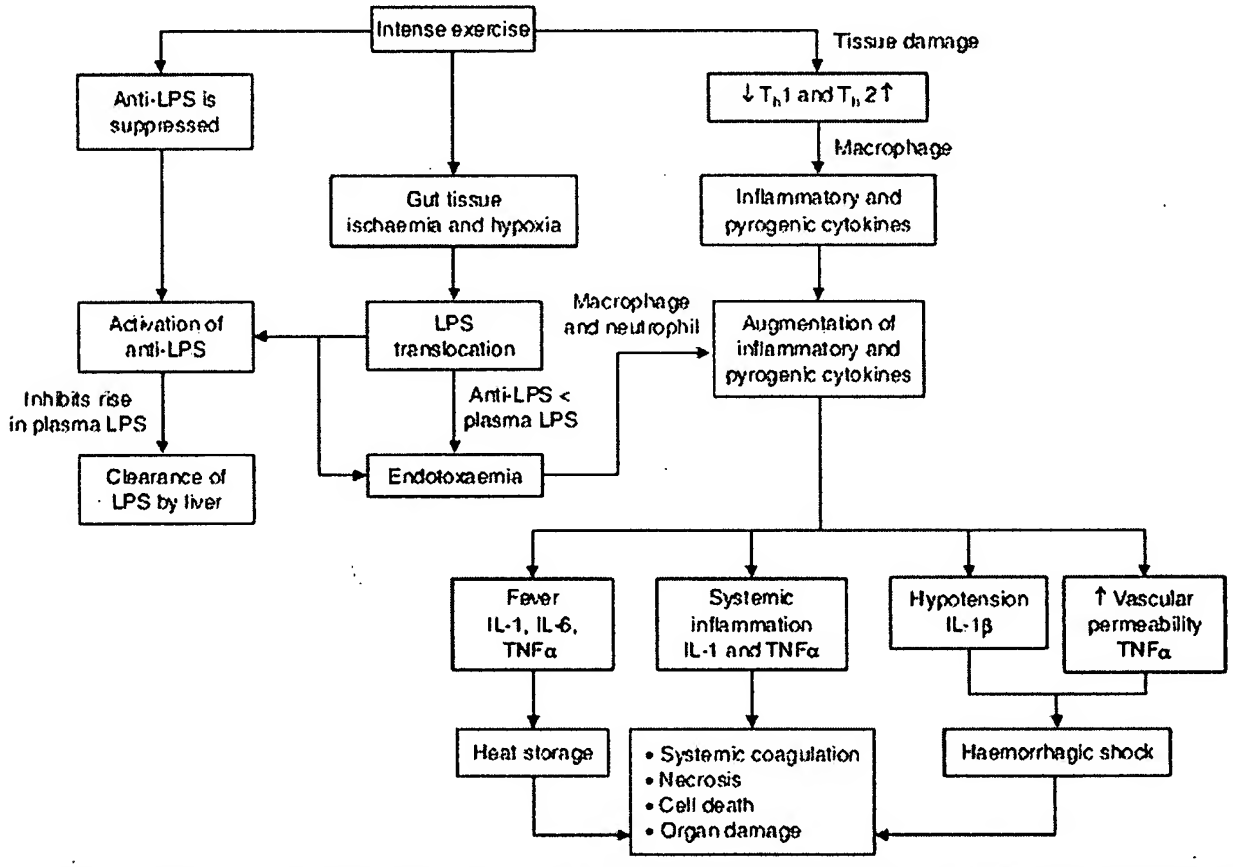


Fig. 2. Cytokine and lipopolysaccharide responses during intense exercise. Intense exercise suppresses anti-lipopolysaccharide antibodies (anti-LPS) and T-helper cell 1 (T_h1) immunity, and promotes LPS translocation and T-helper cell 2 (T_h2) immunity. The suppression of anti-LPS facilitates the accumulation of LPS in the circulation, which induces an increase in inflammatory and pyrogenic cytokines. The suppression of T_h1 and promotion of T_h2 immunity, along with exercise-induced muscle tissue damage also induce the synthesis and release of inflammatory and pyrogenic cytokines. These cytokines promote pyrogenesis, systemic inflammation, increased vascular permeability and hypotension, which contribute to the symptoms of heat stroke. IL = interleukin; TNFα = tumour necrosis factor-α.

50. Endotoxemia. Endotoxin is a potent inducer of neutrophils and monocytes, which, in turn, produce cytokines, including TNF-α, IL-6, and IL-1;TNF release also induces IL-1 synthesis. IL-1 and TNF-α promote inflammation and induce fever. The synthesis and actions of IL-1 and TNFα are counter-regulated by anti-inflammatory cytokines, such as IL-10; IL-1ra, sTNF-r1 and -r2, and IL-6. Heat stroke increases the concentrations of pro- (IL-1 and TNFα) and anti-inflammatory cytokines (IL-6, IL-1ra, and IL-10) in humans and animals. This concomitant increase in both classes of cytokines indicates activation of conflicting immune processes. Whereas inflammatory cytokines help control endotoxemia, their activation in the central circulation leads to other complications such as systemic inflammation and coagulation.

At the same time, inhibiting the activities these cytokines allows endotoxemia to progress. During heat stroke, the body's systems act to counteract the immediate threat of endotoxemia, favoring the pro-inflammatory cytokines, and sepsis, systemic inflammation, DIC and MOF. This sequence of systemic inflammation highlights the consequences when a local inflammatory response occurs in the central circulation. Whereas local inflammation promotes tissue repair, the activation of inflammatory cytokines in the circulation leads to systemic inflammation eventuating in shock. Exercise-induced immune suppression has been attributed to a shift in the balance between the T cell subsets (Th1 and Th2 cells). Th1 cells function primarily in cell-mediated immunity, promoting phagocytic activities against intracellular pathogens. Th2 cells function in humoral immunity and are closely associated with B cells. Intense endurance exercise suppresses Th1 immunity, but does not change Th2 immunity. The suppression of Th1 immunity may be specific to activities involving long-distance running, which causes more physical trauma and damage to the intestines and muscle tissues than cycling.

51. Immunity in Exercise and Heat Stroke. Disturbance of immune function during heat stroke shares some common aspects with that arising in response to intense exercise (Figure 3). Such common responses include endotoxemia, up-regulation or over-expression of pro-inflammatory cytokines occurring under heat stress is the primary pathway for heat stroke. This model is based on exposure to multiple bouts of exercise over an extended time period; chronic and acute immune disturbances act synergistically with each bout of intense exercise to compound the risk of heat stroke. (Lim CL, Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. Sports Med 2006; 36:39-640).

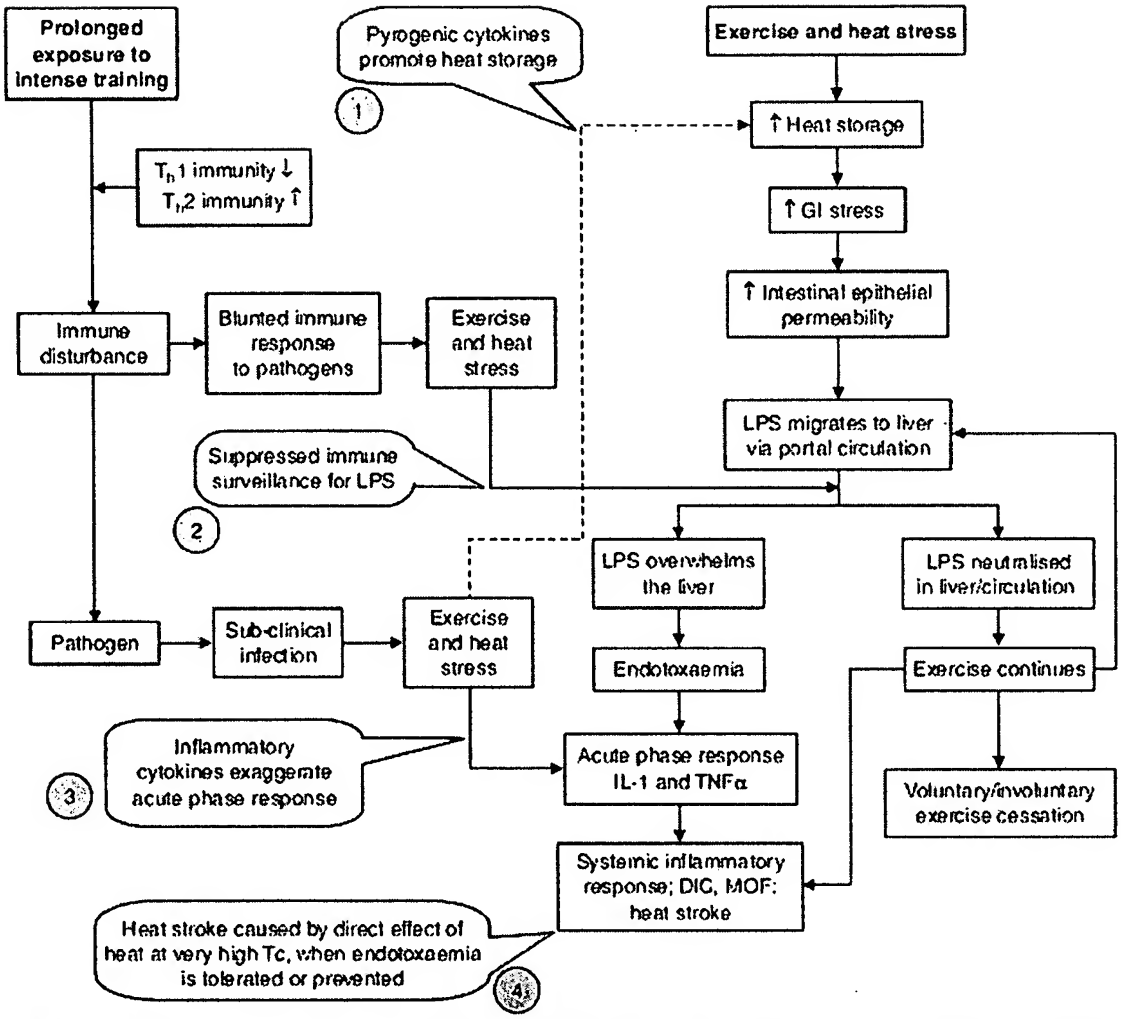


Fig. 3. The dual pathway model of heat stroke. Right side of model (thermoregulation component): exposure to intense exercise increases heat storage and permeability of gut epithelial tissue. This promotes lipopolysaccharide (LPS) translocation from the gut into the portal circulation, which transports the LPS to the liver to be neutralised and removed from the body. Exercise continues until voluntary cessation or exhaustion if the anti-LPS system is able to cope with the magnitude of LPS translocation. When the liver is overwhelmed by LPS, LPS leaks into the central circulation, resulting in endotoxaemia, which can trigger the acute phase response, resulting in heat stroke. Left side of model (immune-regulation component): prolonged exposure to intense exercise suppresses cell-mediated T-helper cell 1 (T_h1) immunity, reduces the immune response to pathogens, and increases the risk of an infection. These exercise-induced immune disturbances can contribute to heat storage and endotoxaemia by promoting pyrogenesis (1) and inflammation (3), and suppressing immune surveillance against LPS (2). When endotoxaemia can be tolerated or prevented, continuing exercise and heat exposure will elevate Tc to a point where heat stroke can occur via the direct effect of heat on tissue cells (4). DIC = disseminated intravascular coagulation; GI = gastrointestinal; IL = interleukin; MOF = multi-organ failure; Tc = core temperature; T_h2 = T-helper cell 2; TNFα = tumour necrosis factor-α; ↓ indicates decrease; ↑ indicates increase.

52. Delayed Onset Muscle Soreness (DOMS). Exercise-induced muscle damage is a common phenomenon resulting from the performance of unaccustomed exercise or exercise with an increased intensity or duration. The well documented symptoms of muscle damage include disruption of intracellular muscle structure, sarcolemma and extracellular matrix, prolonged impairment of muscle function, and delayed-onset muscle soreness (DOMS), stiffness and swelling. A particular component of exercise, eccentric muscle action, is the principle factor responsible for muscle damage. Active muscles may be referred to as performing isometric (constant length), concentric (shortening) or eccentric (lengthening) actions. Eccentric

muscle actions result in greater evidence of muscle damage than isometric or concentric actions. These forms of eccentric muscle action rarely occur in isolation in natural human movement. Instead, natural muscle function occurs in a sequence of active eccentric action followed by an active concentric action, known as the stretch-shortening cycle (SSC). This natural form of muscle function is utilized when body segments are subjected to impact or stretch, due to external forces such as gravity, and is utilized in non-sporting functional activities and most sporting activities such as, running, jumping, throwing and weightlifting. The SSC has a well recognized purpose: enhancement of performance during the final propulsive (concentric) action when compared with the performance of an isolated concentric action. Running downhill increases the contribution of eccentric actions to performance and is a greater stimulus for damage than level or uphill running. Intense or prolonged running, plyometrics and resistance exercise are inherent components of training and competition for most athletes. Moreover, exercise-induced muscle damage occurs frequently in athletic populations, especially during periods of overreaching or overtraining.

53. Muscle soreness is the most commonly used marker of exercise-induced muscle damage in human studies is probably the most well recognized indicator of damage among athletic populations, and yet shares a poor temporal relationship with histological evidence of muscle damage and measures of muscle function after intense eccentric exercise, a person will go to bed with only minor discomfort but will wake the next morning with severe, and in some cases almost disabling pain, first appreciated when trying to get out of bed. All discomfort usually subsides within 96 hours. Thus, the term 'DOMS' is appropriate in describing the typical time course of the sensation but conveys little about the nature of the sensation. The sensation of soreness comprises muscle tenderness, pain on palpation, and also mechanical stiffness in the muscle that results in pain when the muscle is passively stretched or activated. Functional impairments (e.g. reductions in strength and power) are immediate, prolonged, and perhaps the most important symptom of damage when considering athletic performance in the presence of muscle damage. Although many types of treatment have been recommended for DOMS, none have had major success and the symptoms subside spontaneously. It should be noted that DOMS describes a symptom complex of delayed pain for muscle damage due to eccentric exercise that is generally self-limiting whereas muscle damage from intense, long duration exercise with or without thermal stress causes immediate symptoms and signs (Aoi W, Naito Y, Takanami Y, Kawai Y, Sakuma K, Ichikawa H et al. Oxidative stress and delayed-onset muscle damage after exercise. Free Radic Biol Med 2004; 37:480-487; Byrne C, Twist C, Eston R. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. Sports Med 2004; 34:49-69; Cheung K, Hume P, Maxwell L. Delayed onset muscle soreness : treatment strategies and performance factors. Sports Med 2003; 33:145-164; Maddali S, Rodeo SA, Barnes R, Warren RF, Murrell GA. Postexercise increase in nitric oxide in football players with muscle cramps. Am J Sports Med 1998; 26(6):820-824). Treatment with the motion platform speeds up repair by scavenging free oxygen and nitrogen radicals, and reducing nuclear factor kappa beta activity.

54. Performance Impairment. Eccentric exercise resulting in the symptoms of exercise-induced muscle damage results in a well documented reduction in isometric and dynamic strength. Immediate and prolonged reductions in power-generating ability have also been observed during maximal cycling and vertical jump movements, suggesting that

performance decrements observed in the laboratory are likely to transfer to an applied athletic setting. Prolonged losses of strength and power, impaired neuromuscular control, selective type II fibre damage and reflex inhibition are documented outcomes of muscle damage that have the potential to adversely affect dynamic, multi-joint movements that are associated with athletic activity. The ability for the muscle to generate power is reduced for about three days and interferes with performance of endurance runs and to a lesser extent with intermittent sprint performance (Byrne C, Twist C, Eston R. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. Sports Med 2004; 34:49-69; Marcora SM, Bosio A. Effect of exercise-induced muscle damage on endurance running performance in humans. Scand J Med Sci Sports 2007, in press).

55. **Basis for Utility of Claim 21.** The motion platform applied for 45 minutes produces whole body periodic acceleration that increases pulsatile shear stress to the endothelium which in turn causes release of increased amounts of nitric oxide (NO) into the circulation from activation of endothelial nitric oxide synthase (eNOS). In addition, eNOS is upregulated eNOS for at least 24 hours. The increased nitric oxide and upregulation of eNOS as well as increase in other mediators released by shear stress such as prostacyclin and adrenomedullin form the basis for Claim 21. Such treatment before, during, or after athletic performance prevents and/or treats tissue damage, reduces systemic stress, increases athletic performance, and/or prevents/treats any of the problems caused by strenuous athletic activity. Strenuous exercise has potential to damage such tissues as skeletal muscle, myocardium, gastrointestinal tract and liver. Such damage is related to oxidative stress, ischemia/reperfusion injury and inflammation. We have shown as have others that small amounts of NO released into the circulation from activation of eNOS prevent or minimize this damage by 1) preconditioning , conditioning, and postconditioning the heart, skeletal muscle, gastrointestinal tract and the liver as well as other tissues when these events take place. Small amounts of nitric oxide released from increased activity of eNOS through the action of the motion platform act as a direct antioxidant with antiflammatory properties through suppression of nuclear factor kappa beta, the key transcription factor regulating the inflammatory response and cell adhesion molecules. Treatment with the periodic acceleration that release of NO and prostacyclin from the endothelium minimizes propensity to upper respiratory tract infections and inflammation by suppression of nuclear factor kappa beta and scavenging of free oxygen and nitrogen radicals. Bibliography for the paragraph is printed immediately below.

Bibliography for Preceding Paragraph 55.

- Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752;
- Adams JA, Mangino MJ, Bassuk J, Kurlansky P, Sackner MA. Regional blood flow during periodic acceleration. Crit Care Med 2001; 29:1983-1988;
- Adams JA, Bassuk J, Wu D, Kurlansky P. Survival and normal neurological outcome after CPR with periodic Gz acceleration and vasopressin. Resuscitation 2003; 56:215-221;
- Adams JA, Bassuk J, Wu D, Grana M, Kurlansky P, Sackner MA. Periodic acceleration: effects on vasoactive, fibrinolytic, and coagulation factors. J Appl Physiol 2005; 98(3):1083-1090;

- Adams JA, Bassuk J, Kurlansky P, Coelho J, Wu D. Conditioning the endothelium during CPR. Wolf Creek Meeting on Cardiopulmonary Resuscitation, abstract, 2005;
- Adams JA, Bassuk J, Wu H, Arias J, Wu D, Jarapur V et al. Preconditioning with periodic acceleration (pGz) prior to whole body ischemia reperfusion injury ameliorates myocardial stunning and arrhythmias. Circulation, in press, 2007;
- Adams JA, Bassuk J, Wu H, Arias J, Wu D, Jarapur V et al. Post treatment with periodic acceleration (pGz) after cardiac arrest decreases acute post resuscitation myocardial stunning. Circulation, in press, 2007;
- Badhwar A, Bihari A, Dungey AA, Scott JR, Albion CD, Forbes TL et al. Protective mechanisms during ischemic tolerance in skeletal muscle. Free Radic Biol Med 2004; 36:371-379;
- Brell B, Hippenstiel S, David I, Pries AR, Habazettl H, Schmeck B et al. Adrenomedullin treatment abolishes ileal mucosal hypoperfusion induced by Staphylococcus aureus alpha-toxin--an intravital microscopic study on an isolated rat ileum. Crit Care Med 2005; 33:2810-2816;
- Lefer AM, Lefer DJ. The role of nitric oxide and cell adhesion molecules on the microcirculation in ischaemia-reperfusion. Cardiovasc Res 1996; 32:743-751;
- Lim CL, Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke : the dual pathway model of heat stroke. Sports Med 2006; 36:39-64;
- Nava G, Adams JA, Bassuk J, Wu D, Kurlansky P, Lamas GA. Echocardiographic comparison of cardiopulmonary resuscitation (CPR) using periodic acceleration (pGz) versus chest compression. Resuscitation 2005; 66:91-97;
- Nosaka K, Muthalib M, Lavender A, Laursen PB. Attenuation of muscle damage by preconditioning with muscle hyperthermia 1-day prior to eccentric exercise. Eur J Appl Physiol 2007; 99:183-192;
- Sackner MA, Gummels E, Adams JA. Nitric oxide is released into circulation with whole-body, periodic acceleration. Chest 2005; 127:30-39;
- Sackner MA, Gummels E, Adams JA. Effect of moderate-intensity exercise, whole-body periodic acceleration, and passive cycling on nitric oxide release into circulation. Chest 2005; 128:2794-2803;
- Uryash A, Wu H, Bassuk J, Kurlansky P, Sackner MA, Adams JA. Nitroprusside, L-NAME and whole body, periodic acceleration in rats. Circulation , in press, 2007;
- Uryash A, Wu H, Bassuk J, Kurlansky P, Adams JA, Sackner MA. Whole body periodic acceleration blunts hypertensive action of L-NAME in rats. Circulation, in press, 2007;
- Walford G, Loscalzo J. Nitric oxide in vascular biology. J Thromb Haemost 2003; 1::2112-2118;
- Wang WZ, Fang XH, Stepheson LL, Khiabani KT, Zamboni WA. NOS upregulation attenuates vascular endothelial dysfunction in the late phase of ischemic preconditioning in skeletal muscle. J Orthop Res 2004; 22:578-585;
- Wu D, Bassuk J, Arias J, Peschiera I, Lamet A, Kurlansky P et al. Post-resuscitation reperfusion injury: Comparison of periodic Gz acceleration versus Thumper CPR. Resuscitation 2006; 70:454-462;
- Wu H, Arias J, Bassuk J, Kurlansky P, Adams JA. Periodic acceleration (pGz) increases eNOS and nNOS in healthy rat heart. Circulation, in press, 2007;

- Yamaguchi T, Dayton C, Shigematsu T, Carter P, Yoshikawa T, Gute DC et al. Preconditioning with ethanol prevents postischemic leukocyte-endothelial cell adhesive interactions. Am J Physiol Heart Circ Physiol 2002; 283:H1019-H1030; and
- Zardi EM, Dobrina A, Amoroso A, Afeltra A. Prostacyclin in liver disease: a potential therapeutic option. Expert Opin Biol Ther 2007; 7(6):785-790.

Claim 22

56. Claim 22 recites "wherein regular treatment with periodic acceleration as a regimen for the athlete prevents and/or treats tissue damage, reduces systemic stress, increases athletic performance, and/or prevents/treats any of the problems caused by strenuous athletic activity". Most of the justification for this claim is covered under claim 21. Specifically, this claim asserts that regular treatment will have the same benefits as the episodic treatments as above. This has the basis in chronic preconditioning. In preconditioning, organs can be protected for a matter of hours after an agent used for preconditioning is administered a single time but if administration is repeated over days, then the effects can last 24 hours or longer. This was found to be the case for protection against the deleterious effects of myocardial ischemia (Wang Y, Ahmad N, Wang B, Ashraf M. Chronic preconditioning: a novel approach for cardiac protection. Am J Physiol Heart Circ Physiol 2007; 292:H2300-H2305). In studies carried out in our laboratory, one days treatment with the motion platform blunted the asthmatic and inflammatory responses in asthmatic sheep but failed to reduce airways hyperreactivity 24 hour post allergen challenge. However, seven treatments on four days completely abolished airways hyperreactivity 24 hours after allergen challenge.

57. **Basis for Utility of Claim 22.** Chronic preconditioning with motion platform produces long term protection against adverse effects of ischemia and inflammation in the heart and lungs, respectively. Therefore, it would be expected to prevent and/or treat tissue damage, reduce systemic stress, increase athletic performance, and/or prevent/treat any of the problems caused by strenuous athletic activity.

Claim 23

58. Claim 23 recites "wherein pretreatment with periodic acceleration improves athletic performance by preconditioning a body tissue of the athlete". Most of the justification for this claim is covered under Claim 21. Nitric oxide as released from eNOS through periodic acceleration scavenges free oxygen and nitrogen radicals and suppresses activity of vascular NADPH oxidases to minimize oxidative stress which damages skeletal and cardiac muscle (Guzik TJ, Harrison DG. Vascular NADPH oxidases as drug targets for novel antioxidant strategies. Drug Discov Today 2006; 11:524-533; Walford G, Loscalzo J. Nitric oxide in vascular biology. J Thromb Haemost 2003; 1:2112-2118). Preconditioning with periodic acceleration inhibits activation of nuclear factor kappa beta that in turn suppresses inflammation in skeletal muscle. Increased activation of eNOS with periodic acceleration prior to the athletic event prevents ischemic damage to skeletal and cardiac muscle as well as gastrointestinal mucosa. eNOS activation has anti-inflammatory effects on skeletal muscle. Mitigation of such deleterious events that may occur with strenuous exercise improves athletic performance.

59. **Basis for Utility of Claim 23.** Preconditioning with the motion platform protects against adverse effects of oxidative stress, ischemia, and inflammation thereby promoting optimal athletic performance.

Claim 24

60. Claim 24 recites "wherein pretreatment with periodic acceleration mitigates skeletal muscular cramps and/or helps prevent muscle strains during an athletic event". Skeletal muscle cramps during athletic activities relate to involuntary shortening of the affected muscle that is placed into a prolonged contracted state. It is preceded by muscle fatigue (Schwellnus MP. Muscle cramping in the marathon : aetiology and risk factors. Sports Med 2007; 37:364-367). The latter occurs as a result of increased oxidative stress associated with athletic performance (Finaud J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. Sports Med 2006; 36:327-358). Oxidative stress is associated with increased activity of nuclear factor kappa beta (Gomez-Cabrera MC, Martinez A, Santangelo G, Pallardo FV, Sastre J, Vina J. Oxidative stress in marathon runners: interest of antioxidant supplementation. Br J Nutr 2006; 96 Suppl 1:S31-S33). Oxidative stress also reduces taurine levels, an amino acid with antioxidant properties. Skeletal muscles of patients with liver fibrosis and muscular cramps show reduction of taurine levels. Muscular cramps in such patients are ameliorated with oral taurine administration (Miyazaki T, Matsuzaki Y, Ikegami T, Miyakawa S, Doy M, Tanaka N et al. The harmful effect of exercise on reducing taurine concentration in the tissues of rats treated with CCl4 administration. J Gastroenterol 2004; 39:557-562). Extremely high levels of serum nitrite in the micromolar range due to activation of iNOS from macrophages and leukocytes has been found in football players with muscle cramps (Maddali S, Rodeo SA, Barnes R, Warren RF, Murrell GA. Postexercise increase in nitric oxide in football players with muscle cramps. Am J Sports Med 1998; 26:820-824). Increased iNOS activity occurs with both oxidative stress and nuclear factor kappa beta activity. iNOS activity is reduced by increased eNOS activity as occurs during treatment with periodic acceleration (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752; Cirino G, Wheeler-Jones CP, Wallace JL, Del SP, Baydoun AR. Inhibition of inducible nitric oxide synthase expression by novel nonsteroidal anti-inflammatory derivatives with gastrointestinal-sparing properties. Br J Pharmacol 1996; 117:1421-1426; Stefano GB, Prevot V, Cadet P, Dardik I. Vascular pulsations stimulating nitric oxide release during cyclic exercise may benefit health: a molecular approach (review). Int J Mol Med 2001; 7:119-129).

61. **Basis for Utility of Claim 24.** Muscle cramps during athletic performance are associated with increased oxidative stress and activated nuclear factor kappa beta. Treatment with the motion platform upregulates eNOS thereby inhibiting these factors which minimizes occurrence of muscular cramps.

Claim 25

62. Claim 25 recites "wherein pretreatment with periodic acceleration mitigates and/or helps prevent delayed onset muscular soreness (DOMS) and involuntary muscle

cramps and spasms immediately following the athletic event and/or delayed until the sleeping hours". The justification for this claim has been covered in Claims 21, 23, and 24. Muscle soreness is the most commonly used marker of exercise-induced muscle damage in human studies is probably the most well recognized indicator of damage among athletic populations, and yet shares a poor temporal relationship with histological evidence of muscle damage and measures of muscle function after intense eccentric exercise, a person will go to bed with only minor discomfort but will wake the next morning with severe, and in some cases almost disabling pain, first appreciated when trying to get out of bed. All discomfort usually subsides within 96 hours. Thus, the term 'DOMS' is appropriate in describing the typical time course of the sensation but conveys little about the nature of the sensation. The sensation of soreness comprises muscle tenderness, pain on palpation, and also mechanical stiffness in the muscle that results in pain when the muscle is passively stretched or activated. Functional impairments (e.g. reductions in strength and power) are immediate, prolonged, and perhaps the most important symptom of damage when considering athletic performance in the presence of muscle damage. Although many types of treatment have been recommended for DOMS, none have had major success and the symptoms subside spontaneously. It should be noted that DOMS describes a symptom complex of delayed pain for muscle damage due to eccentric exercise that is generally self-limiting whereas muscle damage from intense, long duration exercise with or without thermal stress causes immediate symptoms and signs (Aoi W, Naito Y, Takanami Y, Kawai Y, Sakuma K, Ichikawa H et al. Oxidative stress and delayed-onset muscle damage after exercise. Free Radic Biol Med 2004; 37:480-487; Byrne C, Twist C, Eston R. Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. Sports Med 2004; 34:49-69; Cheung K, Hume P, Maxwell L. Delayed onset muscle soreness: treatment strategies and performance factors. Sports Med 2003; 33:145-164; Maddali S, Rodeo SA, Barnes R, Warren RF, Murrell GA. Postexercise increase in nitric oxide in football players with muscle cramps. Am J Sports Med 1998; 26:820-824). Treatment with the motion platform speeds up repair by scavenging free oxygen and nitrogen radicals, and reducing nuclear factor kappa beta activity.

63. **Basis for Utility of Claim 25.** Muscle cramps following athletic performance are associated with increased oxidative stress and activated nuclear factor kappa beta, a condition known as delayed onset muscular soreness (DOMS). Treatment with the motion platform by upregulating eNOS inhibits those factors that account for symptoms of DOMS.

Claim 26

64. Claim 26 recites "wherein pretreatment with periodic acceleration is used to treat exercise-induced bronchospasm in an athlete". The prevalence of exercise induced bronchospasm (EIB) in the general population is unknown. However, it is generally considered that 80 to 90% of individuals with asthma, 40% of those with allergic rhinitis, and 12 to 15% of the general population experience EIB with moderate exercise. It is likely that all individuals with asthma will experience EIB if the exercise intensity and duration are sufficient. In a survey of 1984 Olympic athletes, approximately 11% of US athletes experienced EIB. A survey of the 1998 US Winter Olympic Team found that the overall incidence of EIB across all sports and genders was 23%. The highest incidence of EIB was found in cross-country skiers, where 50%

of the athletes (female = 57%; male = 43%) were diagnosed with EIB. Across seven sports evaluated, the prevalence of EIB among the female and male athletes was 26 and 18%, respectively. In a screening study of 214 high school football players, 9% demonstrated EIB. Correspondingly, in a screening study of 166 middle and high school athletes, 13% demonstrated EIB. The incidence of EIB has been reported to be as high as 19.3% of Australian school children. Therefore, EIB is a significant finding in both asthmatic and non-asthmatic populations (Gotshall RW. Exercise-induced bronchoconstriction. Drugs 2002; 62:1725-1739).

65. The stimulus by which exercise causes an attack of asthma is the loss of water by vaporation from the surface of the airways in bringing the air inspired to body conditions. This loss of water causes dehydration and cooling of the airway surface. When water loss is prevented, by breathing warm humid air, exercise does not provoke an attack of asthma. The mechanism whereby dehydration causes the airways to narrow is the release of inflammatory mediators in response to an increase in osmolarity of the airway surface. When exercise provokes an attack of asthma the bronchial smooth muscle is likely to have been stimulated by a variety of inflammatory mediators including prostaglandins, leukotrienes and histamine. The primary source of these mediators is the mast cell found on and just below the airway surface. The importance of these contractile mediators in clinically recognized asthmatics has been appreciated ever since the mast-cell-stabilizing agent sodium cromoglycate was shown to inhibit the attack of asthma caused by exercise 35 years ago. At that time histamine was considered to be the major candidate but now prostaglandins and leukotrienes are recognized as more important than histamine, not only for determining the severity of the attack of asthma provoked by exercise but also for the time for which it is sustained. Histamine appears to be important when the exercise stimulus is intense and the smaller airways are involved in the conditioning process. Regular treatment with inhaled corticosteroids reduces the severity of the attack of asthma following exercise. The severity will be reduced over days although months of treatment are usually required for complete resolution. Since corticosteroids have a limited ability to inhibit mast cell mediators, it is more likely that their beneficial effect is to reduce mast cell number and thus concentration of the mediators. (Anderson SD. How does exercise cause asthma attacks? Curr Opin Allergy Clin Immunol 2006; 6:37-42).

66. Whole body periodic acceleration blunts the immediate bronchoconstrictor response to antigen challenge a result of eNOS release of nitric oxide preventing mast cell degranulation in a an analogous action to sodium cromoglycate. eNOS release of NO prevents the late bronchoconstrictor response 6 to 8 hours after the immediate response by suppressing inflammation as assessed by inhibition of nuclear factor kappa beta activity in bronchopulmonary lavage fluid. In contrast to the lengthy treatment time for corticosteroids to achieve results, treatment with periodic acceleration is immediate and chronic treatment over four days will also suppress suppresses airways hyperreactivity (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752). Thus, periodic acceleration could be a standard of care for exercise induced bronchospasm in athletes engaged in competition since no exogenous drugs are required that might be called into question.

67. **Basis for Utility of Claim 26.** Treatment with the motion platform through nitric oxide produced by activation of eNOS prevents release of inflammatory mediators from mast cells that account for bronchospasm associated with exercise.

Claim 27

68. Claim 27 recites "wherein pretreatment with periodic acceleration helps to reduce and/or prevent susceptibility of athletes to viral and bacterial infections".

69. Infections in Elite Athletes. Upper respiratory illness (URI) is the most common medical condition affecting elite athletes. Thirty-two elite and 31 recreationally competitive triathletes and cyclists, and 20 sedentary controls (age range 18.0-34.1 yr) participated in a prospective surveillance study. Nasopharyngeal and throat swabs were collected from subjects presenting with two or more defined upper respiratory symptoms. Thirty-seven URI episodes were reported in 28 subjects. Incidence rate ratios for illness were higher in both the control subjects (1.93, 95% CI: 0.72-5.18) and elite athletes (4.50, 1.91-10.59) than in the recreationally competitive athletes. Infectious agents were identified in only 11 (two control, three recreationally competitive, and six elite) out of 37 illness episodes. Rhinovirus was the most common respiratory pathogen isolated. Symptom and functional impairment severity scores were higher in subjects with an infectious pathogen episode, particularly on illness days 3-4. These results confirm a higher rate of URI among elite athletes than recreationally competitive athletes during this training and competition season (Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG et al. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 2007; 39:577-586). The incidence of symptoms of upper respiratory tract illness is increased in the days following prolonged strenuous endurance events and it has been generally assumed that this is due to the temporary exercise-induced depression of immune function. More recently it has been proposed that at least some of these symptoms are attributable to inflammation of the upper respiratory tract rather than to infectious episodes (Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG et al. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 2007; 39:577-586). In either case, suppression of nuclear factor kappa beta that is elevated in tissues with strenuous exercise (Bar-Shai M, Carmeli E, Reznick AZ. The role of NF-kappaB in protein breakdown in immobilization, aging, and exercise: from basic processes to promotion of health. Ann N Y Acad Sci 2005; 1057:431-447) by NO released from eNOS through the motion platform (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752) should minimize episodes of infection or inflammation. This will enable the athlete to perform at his/her highest level.

70. **Basis for Utility of Claim 27.** Suppression of nuclear factor kappa beta that is elevated in tissues with strenuous exercise by NO released from eNOS through the motion platform minimizes episodes of infection or inflammation. This will enable the athlete to perform at his/her highest level.

Claim 28

71. Claim 28 recites "wherein the pretreatment, treatment, and/or post-treatment with periodic acceleration treats or prevents cramps, aches, soreness, spasms, and other maladies brought on by exercise and/or other athletic activity". The justification for this claim has been covered in the justifications for Claims 20 to 27 and relates to whole body periodic acceleration producing pulsatile shear stress to activate eNOS and other genes within the endothelium to release nitric oxide, prostacyclin, tPA and adrenomedullin into the circulation. These beneficial mediators play a role in the preconditioning, conditioning and post conditioning periods for the potential adverse effects of exercise listed under this claim.

72. **Basis for Utility of Claim 28.** This is based upon the important role that mediators released from the endothelium with motion platform inhibit the adverse effects that may be associated with exercise.

Claim 29

73. Claim 29 recites "wherein treatment using periodic acceleration assists or *reduces* the use of corticosteroids and non-steriodal anti-inflammatory drugs (NSAIDs) in management of pain, injury, muscle soreness, strains, and contusions in athletes". Justification for this claim is covered under the discussion of Claims 20-28. This claim has been modified to use the word *reduces* rather than *replaces*. This claim has utility because whole body periodic acceleration reduces inflammation, thereby reducing the need for drugs in managing inflammatory diseases. Also, there is strong supporting evidence that nitric oxide itself has analgesic properties. This is based upon local injection of acetycholine into a rat paw hypergesia model. Hypergesia was reduced by the injection that acetylcholine through release of nitric oxide from eNOS (Durate ID, Lorenzetti BB, Ferreira SH. Peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. Eur J Pharmacol 1990; 186:289-293). In a comparison of NO-naproxen and naproxen in a rat induced abdominal writhing pain test accomplished with intraperitoneal injections of acetic acid, the NO-naproxen compound was superior in providing analgesia to naproxen alone. Thus, nitric oxide itself has analgesic properties. (Davies NM, Roseth AG, Appleyard CB, McKnight W, Del SP, Calignano A et al. NO-naproxen vs. naproxen: ulcerogenic, analgesic and anti-inflammatory effects. Aliment Pharmacol Ther 1997; 11:69-79).

74. **Basis for Utility of Claim 29.** Application of the motion platform causes release of nitric oxide from eNOS thereby providing anti-inflammatory and analgesic effects and thus demonstrating utility of this claim.

Claim 30

75. Claim 30 recites "wherein the provided periodic acceleration causes release of nitric oxide from the vascular endothelium of the subject through activation of endothelial nitric oxide synthase (eNOS) that in turn scavenges reactive oxygen species thereby diminishing or eliminating oxidative stress". An antioxidant is a substance that helps to reduce the severity of oxidative stress either by forming a less active radical or by quenching the damaging ROS/RNS chain reaction on substrates such as proteins, lipids, carbohydrates or DNA.

Aerobic, anaerobic or mixed training provokes a decrease of oxidative stress, which is caused by an increase of the efficiency of the antioxidant system in response to the supplementary production of ROS and RNS during exercise. The training program must be sufficiently long and intense to trigger a consequent adaptive response of the antioxidant system and a decrease of oxidative stress. This adaptation is more important when the training level of the subjects is low at the beginning of the protocol. This training-induced improvement of the antioxidant status and decrease of oxidative stress are extensively documented in the literature. However, there may be a decrease of antioxidant system efficiency, particularly in high-level athletes subjected to an important training and competitive load with an inappropriate diet. These studies suggest a limit beyond which oxidative stress can increase in excess and cause overtraining. The level of ROS and RNS produced during exercise play an important role not only in the induction of muscular lesions but also in the induction and propagation of post-exercise inflammation, which can increase cellular lesions. These phenomena can disrupt muscular functions and lead to the overtraining syndrome. (Finaud J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. Sports Med 2006; 36:327-358).

76. Most of the justification for the utility of this claim is covered under Claim 21. Nitric oxide as released from eNOS through periodic acceleration scavenges free oxygen and nitrogen radicals and suppresses activity of vascular NADPH oxidases to minimize oxidative stress which damages skeletal and cardiac muscle (Guzik TJ, Harrison DG. Vascular NADPH oxidases as drug targets for novel antioxidant strategies. Drug Discov Today 2006; 11:524-533; Walford G, Loscalzo J. Nitric oxide in vascular biology. J Thromb Haemost 2003; 1:2112-2118). Preconditioning with periodic acceleration inhibits activation of nuclear factor kappa beta that in turn suppresses inflammation in skeletal muscle. Increased activation of eNOS with periodic acceleration prior to the athletic event prevents ischemic damage to skeletal and cardiac muscle as well as gastrointestinal mucosa.

77. Nitric oxide in low plasma concentrations as released from eNOS is an antioxidant since it scavenges reactive oxygen and nitrogen specie (Becker BF, Kupatt C, Massoudy P, Zahler S. Reactive oxygen species and nitric oxide in myocardial ischemia and reperfusion. Z Kardiol 2000; 89 Suppl 9:IX/88-IX/91); Espey MG, Miranda KM, Thomas DD, Xavier S, Citrin D, Vitek MP et al. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. Ann N Y Acad Sci 2002; 962:195-206; Walford G, Loscalzo J. Nitric oxide in vascular biology. J Thromb Haemost 2003; 1(10):2112-2118).

78. **Basis for Utility of Claim 30.** The motion platform causes release of nitric oxide from the vascular endothelium of the subject through activation of endothelial nitric oxide synthase (eNOS) that in turn scavenges reactive oxygen species thereby diminishing or eliminating oxidative stress.

Claim 31

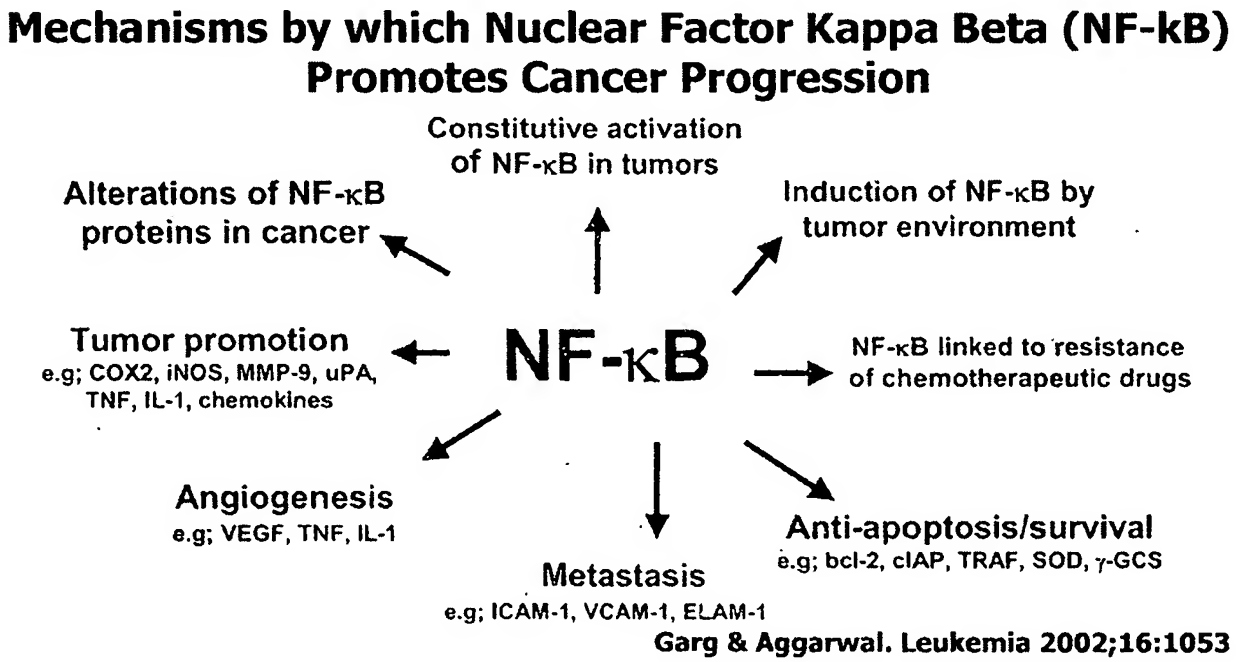
79. Claim 31 recites "wherein the periodic acceleration provided by a motion platform to the subject causes release of nitric oxide from the vascular endothelium of the patient through activation of endothelial nitric oxide synthase (eNOS) which in turn suppresses the

activity of inducible nitric oxide synthase (iNOS)". iNOS activity is reduced by increased eNOS activity as occurs during treatment with periodic acceleration (Cirino G, Wheeler-Jones CP, Wallace JL, Del SP, Baydoun AR. Inhibition of inducible nitric oxide synthase expression by novel nonsteroidal anti-inflammatory derivatives with gastrointestinal-sparing properties. Br J Pharmacol 1996; 117:1421-1426; Stefano GB, Prevot V, Cadet P, Dardik I. Vascular pulsations stimulating nitric oxide release during cyclic exercise may benefit health: a molecular approach (review). Int J Mol Med 2001; 7:119-129). Further, NO from eNOS released during periodic acceleration suppresses nuclear factor kappa beta which increases iNOS activity (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752).

80. **Basis for Utility of Claim 31.** The support for utility of this claim is based upon the known effects of increased eNOS activity in suppressing iNOS activity.

Claim 32

81. Claim 32 recites "wherein the periodic acceleration treats and/or prevents cramps, aches, soreness, spasms, and the like at least because the suppression of nuclear factor kappa beta diminishes IL-1beta, IL-6, tumor necrosis factor and other inflammatory cytokines and adhesion molecules". The figure below is targeted toward cancer progression but is relevant to Claim 32 since it depicts inflammatory cytokines activated by nuclear factor kappa beta activity (Garg A, Aggarwal BB. Nuclear transcription factor-kappaB as a target for cancer drug development. Leukemia 2002; 16:1053-1068).



82. The justification for the utility of claim 32 is, in a large part, described in the above discussion of Claim 21. Additional information is described below.

83. Inflammation and Neutrophil Endothelial Interaction. Strenuous exercise can produce inflammation in muscles that lead to muscle damage. This is also associated with activation of nuclear factor kappa beta, the key transcription factor regulating the inflammatory response (Bar-Shai M, Carmeli E, Reznick AZ. The role of NF-kappaB in protein breakdown in immobilization, aging, and exercise: from basic processes to promotion of health. Ann N Y Acad Sci 2005; 1057:431-447). If inflammation is regarded as the proliferation of white blood cells after soft tissue injury, then the cellular inflammatory response actually begins at the onset of exercise, when the circulating level of neutrophils significantly increases. Neutrophils arrive in muscle and affect the host inflammatory response during exercise and soft tissue injury. These cells have both specific and nonspecific defensive mechanisms, some of which are capable of causing additional tissue damage. The mechanism for early neutrophilia in the postexercise state is likely due to a combination of factors. During rest, more than half of the circulating neutrophils are marginated along the endothelial walls of blood vessels. At the onset of exercise, increases in epinephrine, blood flow, and cell-signaling molecules demarginate these neutrophils away from the vessel walls, resulting in their mobilization into the circulation. Demargination allows the neutrophils to enter the circulation and redistribute elsewhere in the body, as needed.

84. Recruitment of Neutrophils and Mast Cells. The movement of a neutrophil from the circulation into the tissue, called diapedesis, is under tight regulatory control of the underlying tissue. In skeletal muscle, diapedesis can occur rapidly during exercise. Neutrophil recruitment is ultimately the responsibility of the muscle fibers (myocytes) together with mast cells from a variety of tissues, including the local connective tissue. If a myocyte is perturbed in some fashion, such as in the case of an active stretch or contusion, it communicates with the endothelial wall of the adjacent blood vessel, initiating a cascade of signaling events and resulting in diapedesis. This intercellular communication is accomplished, in part, by a series of cell-signaling molecules, or cytokines.

85. Cytokines. All nucleated cells in the body produce cytokines and similarly express cytokine receptors on their surface membranes. Cytokines act at the surface of the target cells, principally to alter cell function. Skeletal muscle continually produces cytokines in an effort to maintain homeostasis and to regulate function. Simple perturbations of skeletal muscle, such as an active stretch during eccentric exercise, markedly increase the expression of interleukin-1ß, (IL-1ß) and tumor necrosis factor–α (TNF-α). These proinflammatory cytokines upregulate the expression of endothelial leukocyte adhesion molecules (E-selectin) within the endothelium of the adjacent blood vessels. Activation of the endothelium can result in the release of additional IL-1ß, as well as additional proinflammatory cytokines, including IL-6 and IL-8, both of which have been shown to attract neutrophils. Thus, endothelial activation serves 2 purposes: encouraging the adhesion of neutrophils at the site of cell stress (margination) and assisting the cell in recruiting additional neutrophils. (Butterfield TA, Best TM, Merrick MA. The dual roles of neutrophils and macrophages in inflammation: a critical balance between tissue damage and repair. J Athl Train 2006; 41:457-465).

86. Membrane Disruption, Neutrophils, Cytokines. Membrane disruption is probably the initial, propagating event in muscle injury owing to the high forces transmitted during eccentric exercise. In this manner, membrane disruption causes neutrophils to migrate to the damaged area in order to remove the damaged tissue through phagocytosis. Exacerbation of injury can take place after eccentric exercise, including observations that the initial injury is often followed by a secondary loss of muscle force, due to additional but delayed damage to the muscle fibers. This so-called secondary damage has been proposed to be caused by invading neutrophils, potentially due to a second burst of neutrophilia within 24 hours of cessation of exercise. More importantly, this secondary burst appears to mediate damage through the release of cytotoxic compounds. This secondary response likely results from bone marrow release of neutrophils in response to elevated blood catecholamine levels. These neutrophils appear to be more oxidatively active than the first group of neutrophils emigrating to the extracellular matrix (ECM). After muscle injury, myocytes and other cells release cytokines, such as IL-1ß, IL-6, IL-8, and TNF-α, that activate neutrophils to produce a host of cytotoxic substances, including ROS, such as superoxide anions, hypochloride, and hydrogen peroxide. The cytokines IL-1ß, IL-6, and TNF-α all stimulate pathways that contribute to activation of the enzyme NADPH oxidase in neutrophils and endothelium, which generates a ''respiratory burst'' and the subsequent release of reactive oxygen species.

87. **Basis for Utility of Claim 32.** Since nuclear factor kappa beta is the key transcription factor regulating the inflammatory response, its inhibition with nitric oxide as released with periodic acceleration, will treat and prevent the muscle damage and associated symptoms cramps, aches, soreness, spasms, and the like.

Claims 33-34

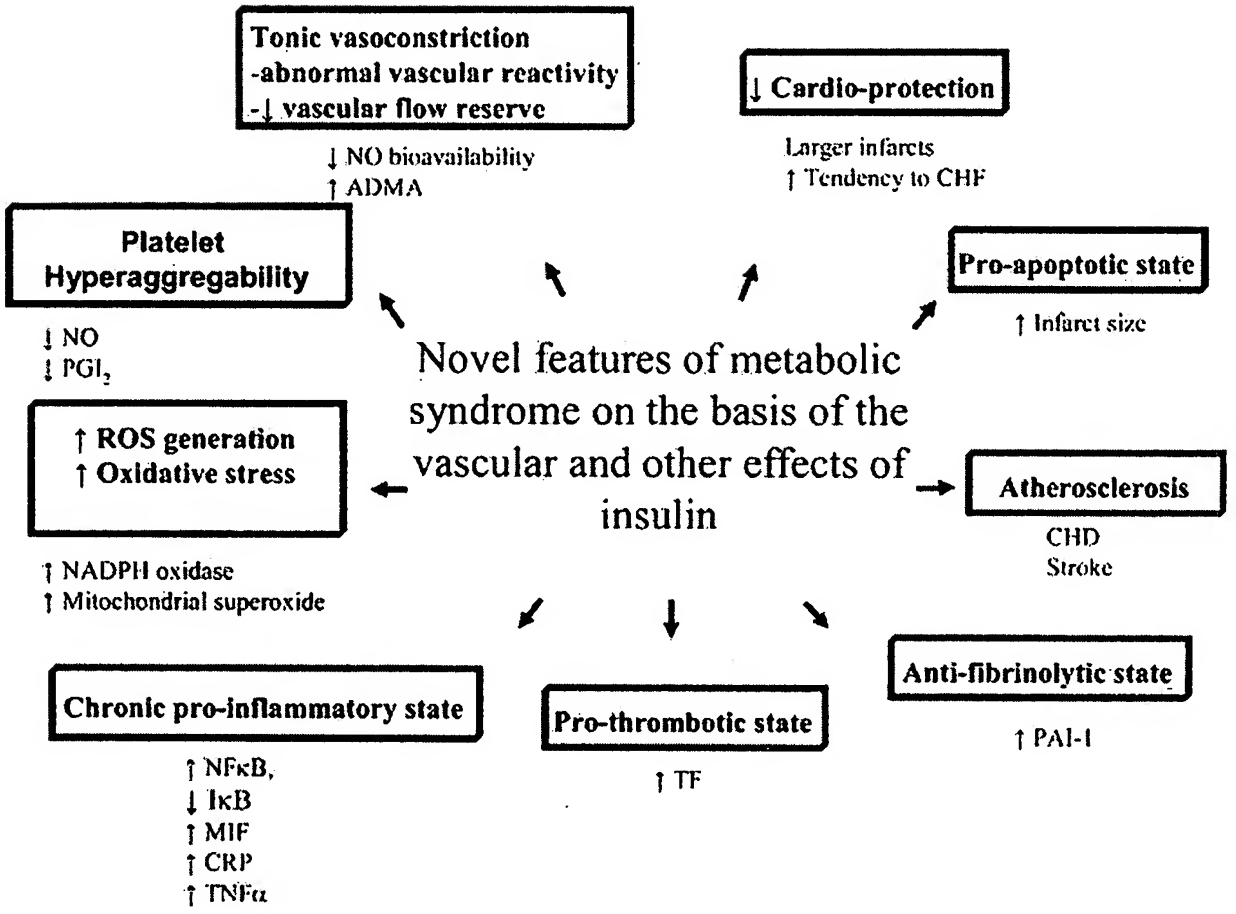
88. Claims 33-34 are canceled.

Claim 35

89. Claim 35 is amended to recite "wherein treatments of periodic acceleration are used to ameliorate metabolic syndrome, ~~to improve sports performance, and/~~or to improve skeletal muscle pathology associated with the cachexia of COPD and cancers in weight control of the subject". This is because sports performance has already been covered with its synonym, athletic performance in Claim 21. The motion platform of claim 20, wherein treatment with periodic acceleration before, during, or after *athletic performance* prevents and/or treats tissue damage, reduces systemic stress, *increases athletic performance*, and/or prevents/treats any of the problems caused by strenuous athletic activity.

90. Metabolic Syndrome. The original description of the Metabolic Syndrome consisted of obesity, insulin resistance, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia and dyslipidemia characterized by elevated triglyceride, and low HDL concentrations. All of the features described above are risk factors for atherosclerosis, and thus, the Metabolic Syndrome constituted a significant risk for coronary heart disease. The proinflammatory state of obesity and metabolic syndrome originates with excessive caloric intake and is probably a result of overnutrition in a majority of patients in the United States. The

proinflammatory state induces insulin resistance, leading to clinical and biochemical manifestations of the metabolic syndrome. This resistance to insulin action promotes inflammation further through an increase in FFA concentration and interference with the anti-inflammatory effect of insulin. The inflammatory aspects of the Metabolic Syndrome and the factors associated with insulin resistance are depicted in the figure below (Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005; 111:1448-1454). The abnormalities depicted in this figure also can be attributed to endothelial dysfunction, e.g., tonic vasoconstriction, platelet hyperaggregability, increased oxidative stress, chronic pro-inflammatory state, pro-thrombotic state, anti-fibrinolytic state, pro-thrombotic state, anti-fibrinolytic state, atherosclerotic state, pro-apoptotic state, and impaired cardioprotective state. In endothelial dysfunction as occurs in the Metabolic Syndrome, eNOS becomes uncoupled because of deficiency in the co-factor tetrahydropterin (BH4) causing production of superoxide from the L-Arginine substrate rather than nitric oxide. This leads to increased oxidative stress. Of interest is that mice with an absent eNOS gene have many features of the Metabolic Syndrome such as hypertension, insulin resistance, dyslipidemia, increased fibrinogen and impaired glucose tolerance (Cook S, Hugli O, Egli M, Vollenweider P, Burcelin R, Nicod P et al. Clustering of cardiovascular risk factors mimicking the human Metabolic Syndrome in eNOS null mice. Swiss Med Wkly 2003; 133:360-363). As depicted in the figure below, prostacyclin and tPA release are diminished and NAPDH oxidase and tissue factor are increased. Increased pulsatile shear stress with whole body periodic acceleration synthesizes BH4 thereby coupling eNOS to produce nitric oxide not superoxide. Pulsatile shear stress achieved with the motion platform promotes release of NO, prostacyclin, tPA, and tissue factor pathway inhibitor thereby blunting the adverse effects of the Metabolic Syndrome depicted below (Adams JA, Bassuk J, Wu D, Grana M, Kurlansky P, Sackner MA. Periodic acceleration: effects on vasoactive, fibrinolytic, and coagulation factors. J Appl Physiol 2005; 98:1083-1090; Lam CF, Peterson TE, Richardson DM, Croatt AJ, d'Uscio LV, Nath KA et al. Increased blood flow causes coordinated upregulation of arterial eNOS and biosynthesis of tetrahydrobiopterin. Am J Physiol Heart Circ Physiol 2006; 290:H786-H793; Westmuckett AD, Lupu C, Roquefeuil S, Krausz T, Kakkar VV, Lupu F. Fluid flow induces upregulation of synthesis and release of tissue factor pathway inhibitor in vitro. Arterioscler Thromb Vasc Biol 2000; 20:2474-2482).



91. Although these factors may be the most important factor in a majority of patients with metabolic syndrome, it is possible that other factors, such as genetic factors, may also contribute to the inflammatory stress in Metabolic Syndrome. Because excessive nutritional intake probably accounts for the inflammation in obesity-associated Metabolic Syndrome, the most rational way to suppress such inflammation is through caloric restriction. The other lifestyle change that affects inflammation is exercise. Exercise results in a fall in the indices of inflammation, such as plasma CRP concentration. The mechanism underlying this effect of exercise is not known; however, it is noteworthy that lifestyle change is a very effective way to reduce the rate of development of diabetes in a prediabetic population, as shown by the diabetes prevention study. Both a reduction in macronutrient intake and exercise cause a reduction in inflammation (Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005; 111:1448-1454).

92. Since the Metabolic Syndrome is an extreme example of endothelial dysfunction, the treatment recommendations listed above should be instituted. In addition, treatment with the motion platform to increase pulsatile shear stress to the endothelium also blunts or reverses the adverse manifestations of the Metabolic Syndrome.

93. Cachexia. This illness-induced loss of skeletal muscle mass is associated with an increase in morbidity and mortality. Unfortunately, even small and persistent changes in protein synthesis or protein degradation lead to large protein deficits because the rate of protein turnover is so high (240–310 g/day). Skeletal muscle is composed of bundles of fibres bound together by collagen tissue. Each cell (fibre) consists of a membrane, many scattered nuclei lying under this membrane, and cytoplasm containing thousands of myofibrils. Myofibrils consist of myosin and actin proteins that are arranged in sarcomeres; contraction occurs when actin slides towards the centre of the myosin scaffold. Many catabolic disorders such as cancer and COPD are associated with a rise in cytokine production, a response thought to be critical for initiating loss of muscle mass. Cytokines also inhibit the formation of new myofibrils. The key molecule in these studies is the transcription factor, nuclear factor kappa beta. The other element in muscle loss—acceleration of protein degradation—occurs by activation of the ubiquitin proteasome proteolytic system in a variety of cachexia producing disorders (e.g., sepsis, cancer, burn injury) and nuclear factor kappa beta is also the inciting factor. (Mitch WE, Price SR. Transcription factors and muscle cachexia: is there a therapeutic target? Lancet 2001; 357(9258):734-735; Wyke SM, Tisdale MJ. NF-kappaB mediates proteolysis-inducing factor induced protein degradation and expression of the ubiquitin-proteasome system in skeletal muscle. Br J Cancer 2005; 92(4):711-721).

94. Cachexia in COPD. Patients with chronic obstructive pulmonary disease (COPD) often lose weight during the course of their disease. This has important clinical implications because it worsens their prognosis and quality of life. Atrophy of skeletal muscle is the main cause of weight loss in COPD. The precise cellular and molecular mechanisms leading to skeletal muscle atrophy in these patients are unclear. Inactivity, systemic inflammation, oxidative stress, tissue hypoxia, and enhanced skeletal muscle apoptosis have been considered, among others, to be potential pathogenic factors. Systemic inflammation9 and hypoxia are particularly prevalent among COPD patients with low body weight and both are well known inducers of the iNOS through the nuclear transcription factor NF-kB pathway. Activation of NF-kB and upregulation of iNOS occur in the skeletal muscle of COPD patients with low body weight. This constitutes a molecular mechanism leading to cachexia in these patients because iNOS upregulation can cause protein nitrotyrosination and favour protein degradation through the ubiquitin-proteasome pathway, and enhance skeletal muscle apoptosis, an event which occurs in COPD patients with low body weight (Agusti A, Morla M, Sauleda J, Saus C, Busquets X. NF-kappaB activation and iNOS upregulation in skeletal muscle of patients with COPD and low body weight. Thorax 2004; 59:483-487).

95. Nitric oxide released from eNOS through treatment with the motion platform inhibits activity of both nuclear factor kappa beta and iNOS and therefore blunts the progression of cachexia in cancer and COPD. This effect can last much longer than the rime of the motion platform treatment (usually 45 minutes) because of upregulation of eNOS and slow release of nitric oxide from S-Nitrosylated thiols that have combined with nitric oxide (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752; Blais V, Rivest S. Inhibitory action of nitric oxide on circulating tumor necrosis factor-induced NF-kappaB activity and COX-2 transcription in the endothelium of the brain capillaries. J Neuropathol Exp Neurol 2001; 60:893-905; Cirino G, Wheeler-Jones CP, Wallace JL, Del SP,

Baydoun AR. Inhibition of inducible nitric oxide synthase expression by novel nonsteroidal anti-inflammatory derivatives with gastrointestinal-sparing properties. Br J Pharmacol 1996; 117:1421-1426; Fortenberry JD, Owens ML, Chen NX, Brown LA. S-nitrosoglutathione inhibits TNF-alpha-induced NFkappaB activation in neutrophils. Inflamm Res 2001; 50:89-95; Marshall HE, Stamler JS. Inhibition of NF-kappa B by S-nitrosylation. Biochemistry 2001; 40:1688-1693; Sackner MA, Gummels E, Adams JA. Nitric oxide is released into circulation with whole-body, periodic acceleration. Chest 2005; 127(1):30-39; Uryash A, Wu H, Bassuk J, Kurlansky P, Sackner MA, Adams JA. Nitroprusside, L-NAME and whole body, periodic acceleration in rats. Circulation , in press. 2007; Wu H, Arias J, Bassuk J, Kurlansky P, Adams JA. Periodic acceleration (pGz) increases eNOS and nNOS in healthy rat heart Circulation, in press, 2007).

96. **Basis for Utility of Claim 35.** Since the basis for the Metabolic Syndrome is endothelial dysfunction marked by nitric oxide deficiency due to uncoupled eNOS as well as inflammation related to activated nuclear factor kappa beta, increased pulsatile shear stress with the motion platform favors normalization of these factors. In cachexia, upregulation of eNOS with the motion platform inhibits nuclear factor kappa beta and iNOS that are primarily responsible for cachexia and skeletal muscle atrophy in cancer and COPD.

Claim 36

97. Claim 36 is amended to recite "wherein periodic acceleration is used to prevent ventricular remodeling". Myocardial infarction frequently leads to left ventricular (LV) dilatation and associated interstitial fibrosis in the non-infarcted myocardium, a condition called ventricular remodeling. This causes depressed cardiac performance and is an independent determinant of morbidity and mortality after MI. This process is driven by the overexpression of various factors, including proinflammatory cytokines, angiotensin II, and norepinephrine, which have direct pathophysiological effects on cardiac myocytes, noncardiac myocytes, and the extracellular matrix. Although the expression of proinflammatory cytokines may be involved in wound healing after MI, it is believed that the overexpression of cytokines damages cardiac tissue and evokes excess deposition of fibrotic components, even in the non-infarcted myocardium. Nuclear factor-kappa beta (NF-κß) is involved in the pathogenesis of heart failure. Its activation leads to trans-activation of cytokines, chemokines, and matrix metalloproteinases (MMPs), promoting inflammatory and fibrotic responses that participate in the progression of myocardial remodeling. Inflammatory mediators, including those of cytokines, chemokines, and MMPs, may play important roles in the progression of cardiac remodeling. Blockade of NF-κß by inhibition of phosporylation has been carried out in a rat model of MI with prevention of structural remodeling. In a genetically manipulated mouse model with permanent coronary ligation to produce MI, blockade of NF-κß prevented ventricular rupture early after MI and improved survival by ameliorating cardiac dysfunction in the late phase, suggesting that blockade of NF-κß might be a new therapeutic strategy to attenuate ventricular rupture and remodeling after MI (Onai Y, Suzuki J, Maejima Y, Haraguchi G, Muto S, Itai A et al. Inhibition of NF-κB improves left ventricular remodeling and cardiac dysfunction after myocardial infarction. Am J Physiol Heart Circ Physiol 2007; 292:H530-H538; Kawano S, Kubota T, Monden Y, Tsutsumi T, Inoue T, Kawamura N et al. Blockade of NF-kappaB improves cardiac function and survival after myocardial infarction. Am J Physiol Heart Circ Physiol 2006; 291:H1337-H1344).

98. NF-κß activation is involved in the hypertrophic response of cardiomyocytes. For example, its activation is required for hypertrophic growth of cultured primary rat neonatal ventricular cardiomyocytes and for myotrophin-induced cardiac hypertrophy in vitro. Cardiac hypertrophic agonists, such as angiotensin II (ANG II) and endothelin-1, stimulate NF-κß activation, whereas amelioration of ANG II-induced cardiac hypertrophy in vitro is positively correlated with decreased NF-κß activation. Ischemia/reperfusion (I/R) injury activates NF-κß in the myocardium both in vitro and in vivo. Because myocardial hypertrophy is an adaptive response to various stimuli, including myocardial I/R injury and pressure overload, NF-κß activation is also involved in development of cardiac hypertrophy in vivo. Thus, NF-κß activation is increased in hypertrophic hearts induced by aortic banding in rats and that specific inhibition of NF-κß activation by adenovirus-mediated gene transfection of an IκB-α dominant negative mutant into the myocardium attenuates the development of cardiac hypertrophy (Li Y, Ha T, Gao X, Kelley J, Williams DL, Browder IW et al. NF-kappaB activation is required for the development of cardiac hypertrophy in vivo. Am J Physiol Heart Circ Physiol 2004; 287:H1712-H1720).

99. Investigation of spontaneously hypertensive rats indicates that cardiac hypertrophy is partly due to NF-κß activation, that inhibition of NF-κß activity by a pharmacologic inhibitor parallels regression of hypertrophy, and that such regression of hypertrophy is partly due to inhibition of NF-κß activity, independent of hypertension. The relationship between NF-κß activity and cardiac remodeling is causal, not coincidental (Gupta S, Young D, Sen S. Inhibition of NF-kappaB induces regression of cardiac hypertrophy, independent of blood pressure control, in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 2005; 289:H20-H29).

100. A fundamental response to increased biomechanical stress such as pressure overload induced hypertrophy and dilation is cardiomyocyte and chamber hypertrophy. Although this may provide initial salutary compensation to the stress, sustained hypertrophic stimulation becomes maladaptive, worsening morbidity and mortality risks because of congestive heart failure and sudden death. Growing evidence highlights oxidative and nitrosative stresses as important mechanisms for this maladaptation. Oxidative stress occurs when excess reactive oxygen species (ROS) are generated that cannot be adequately countered by intrinsic antioxidant systems. Superoxide can further combine with NO, forming reactive compounds such as peroxynitrite, generating nitroso-redox imbalance. ROS generation is a normal component of oxidative phosphorylation and plays a role in normal redox control of physiological signaling pathways. However, excessive ROS generation triggers cell dysfunction, lipid peroxidation, and DNA mutagenesis and can lead to irreversible cell damage or death.

101. Increased shear stress activates eNOS to normally produce nitric oxide. When exposed to oxidative or nitrosative stress or when deprived of BH4 or L-arginine, eNOS becomes structurally unstable. This is called the uncoupled state in which eNOS produces superoxide rather than normally produced nitric oxide. This change has been linked to the endothelial pathophysiology in hypertension, diabetes, smoking, and atherosclerosis. Similar mechanisms also play a key role in the adverse remodeling resulting from chronic pressure

overload. Hearts exposed to trans-aortic constriction develop marked chamber dilation with decreased eNOS in the myocardium and elevation of oxidative stress. The latter is reduced by half by preincubating myocardial extract with the NOS inhibitor, *N*G-nitro-L-arginine methyl ester, suggesting that ROS were being generated by NOS itself. Similarly, animals genetically lacking eNOS exposed to pressure overload developed more modest and compensated concentric hypertrophy, with little cavity dilation, less interstitial fibrosis, and far less oxidative stress. A major factor that may mediate eNOS uncoupling in pressure-overloaded hearts is a decline in BH4 levels. This is supported by both direct BH4 measurements and findings that BH4 supplementation offsets the hypertrophic/dilative phenotype. Hearts with increased ROS because of uncoupled eNOS have increased MMP activation, which, in turn, degrades extracellular matrix, facilitates left ventricular dilatation and worsens cardiac function. The exact mechanism for BH4 reduction is unknown but could relate to oxidation from increased activity of NAPDH oxidases present in the endothelium as a result of conditions precipitating endothelial dysfunction listed above (Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. Hypertension 2007; 49:241-248). BH4 deficiency can be treated by oral administration of BH4, an expensive drug not yet approved by FDA or by increasing shear stress as with exercise or treatment with the motion platform (Lam CF, Peterson TE, Richardson DM, Croatt AJ, d'Uscio LV, Nath KA et al. Increased blood flow causes coordinated upregulation of arterial eNOS and biosynthesis of tetrahydrobiopterin. Am J Physiol Heart Circ Physiol 2006; 290:H786-H793).

102. The renin-angiotensin-aldosterone system (RAAS) appears to be a prominent stimulator of cardiac remodeling after MI. RAAS is significantly upregulated following MI and there is extensive literature documenting the diverse effects of angiotensin II (Ang II) on cardiomyocytes. Reactive oxygen species (ROS) play as an effector of Ang II-induced cardiac remodeling during chronic heart failure. Ang II-ROS signaling acts as a potent stimulator of cardiomyocyte hypertrophy both in vitro and in vivo. An increase in oxidative stress can trigger factors such as TGF-h, a key player in both hypertrophy and extracellular matrix (ECM) formation within the heart leading to the fibrosis associated with MI. Ang II type 2 receptors (AT2) have been shown to trigger activation of the bradykinin-NO pathway, reducing Ang II-induced fibrosis, and may act to antagonize the actions of AT1 receptors.

103. Nitric oxide (NO) has been shown to block some of the effects of Ang II and is able to suppress the hypertrophic effect associated with Ang II. In addition, non-specific pharmacological blockade of nitric oxide synthase (NOS), the enzyme responsible for producing NO, results in more extensive fibrosis after MI. Mice with heart-targeted overexpression of eNOS show that NO provides a protective effect against the damage induced by ischemia. eNOS deficient mice are more susceptible to the damaging effects of MI than wild type mice.

104. In a rat model of coronary artery ligation, the potential protective role of human endothelial nitric oxide synthase (eNOS) in left ventricular (LV) remodeling after myocardial infarction (MI) by a somatic gene transfer approach was evaluated. Six weeks after MI, the LV end-diastolic pressure, heart weight, LV axis length and cardiomyocyte size were markedly increased in the control group, while eNOS gene delivery significantly reduced these parameters. Control rats developed considerably more fibrotic lesions. eNOS gene delivery significantly reduced collagen accumulation. The cardioprotective effect of NO was

accompanied by reduced NADH and NADPH oxidase activities and superoxide formation, TGF-h1 and p27 levels, JNK activation, NF-κB activation, and caspase-3 activity. Thus, NO plays an important role in attenuating cardiac remodeling and apoptosis after myocardial infarction via suppression of oxidative stress-mediated signaling pathways (Smith RS, Jr., Agata J, Xia CF, Chao L, Chao J. Human endothelial nitric oxide synthase gene delivery protects against cardiac remodeling and reduces oxidative stress after myocardial infarction. Life Sci 2005; 76:2457-2471).

105. **Basis for Utility of Claim 36.** Both oxidative stress and activation of nuclear factor kappa beta play major roles in promoting ventricular remodeling following myocardial infarction and in hypertension. Treatment with the motion platform causes pulsatile shear stress that couples eNOS to generate nitric oxide, reduces oxidative stress and inhibits activation of nuclear factor kappa beta, factors that prevent the deleterious effects of ventricular remodeling.

Claim 37

106. Claim 37 is canceled.

Claim 38

107. Claim 38 recites "wherein periodic acceleration is used to treat and/or prevent complications from coronary bypass surgery". The major complications after coronary bypass surgery are due to ischemia/reperfusion (I/R) injury. Clinically, it is characterized by arrhythmias combined with myocardial and microvascular stunning, often leading to low cardiac output syndrome. Myocardial infarction (MI) may be indistinguishable from I/R injury and their association has been noted in patients with fatal outcome. The morphologic counterpart of I/R is a special form of myocardial necrosis known as contraction band necrosis, which has been present in 31% to 100% of CABG patients with fatal outcome. Reperfusion injury is observed in one fourth of the deaths in association with MI. It occurs more often in patients with preoperative NYHA III symptoms and in those in whom endarterectomy is carried out and the anoxic time of the myocardium was longer. The shorter postoperative survival time indicates the lethal nature of this complication (Weman SM, Karhunen PJ, Penttila A, Jarvinen AA, Salminen US. Reperfusion injury associated with one-fourth of deaths after coronary artery bypass grafting. Ann Thorac Surg 2000; 70:807-812).

108. Myocardial infarction is the major cause of heart failure. Coronary reperfusion therapy with thrombolytic and fibrinolytic agents, coronary angioplasty or coronary artery bypass surgery has become established for the management of acute myocardial infarction. However, restoration of blood flow to previously ischemic myocardium results in injury (Table 1) to viable myocardium and a "mismatch" between flow and recovery of mechanical function even in the absence of irreversible damage. Post-infarction survivors with persistent left ventricular dysfunction are prone to develop heart failure and progressive ventricular remodeling. Since the timing and adequacy of reperfusion are major determinants of functional recovery, every effort is made to achieve as early and as complete reperfusion as possible. Reactive oxygen species (ROS) and oxidative stress are major contributors to

reperfusion injury. Although mammalian cells including cardiomyocytes express superoxide dismutase and catalase which act as scavengers of oxygen free radicals, these systems may be overwhelmed after ischemia/reperfusion. Reperfusion after 2 hours of ischemia is associated with significant necrosis but still limits early and late remodeling. Even early reperfusion results in persistent left ventricular dysfunction and stunning and does not guarantee that function will improve. A priority in is therefore to find adjunctive therapies that limit ischemia-reperfusion injury and speed functional recovery (Jugdutt BI. Nitric oxide and cardioprotection during ischemia-reperfusion. Heart Fail Rev 2002; 7:391-405).

Table 1. *Mechanisms for myocardial stunning and reperfusion injury*

- Increased oxygen derived free radicals
- Decreased antioxidant reserve
- Increased peroxynitrite formation
- Vascular injury
- Uncoupling of excitation-contraction
- Calcium overload
- Impaired metabolism
- Decreased mitochondrial energy production
- Impaired energy utilization by myofibrils
- Impaired sympathetic neural responsiveness
- Decreased sensitivity of myofibrils to calcium
- Impaired perfusion
- Damage to extracellular collagen matrix
- Myocardial hibernation

109. Despite meticulous adherence to presently known principles of surgical myocardial protection using advanced cardioplegic technologies, some patients require inotropic support and/or mechanical assist devices postoperatively, when none was required preoperatively. There is good clinical evidence to support the concept that all patients undergoing CABG have varying degrees of myocardial stunning, occasionally requiring inotropic support, which after abatement over hours or days after surgery have no objective evidence of myocardial infarction. However, there is a significant downside to the use of inotropic agents. The classic physiological experiment on a Langendorf rat heart preparation teaches that increasing doses of isoproterenol will cause myonecrosis as the myocardial oxygen consumption exceeds the heart's capacity to increase coronary blood flow. In addition, there is recent evidence that therapeutic levels of inotropic support in the postischemic heart increases intracellular calcium and subsequent apoptosis resulting in cell death, which is probably accentuated in the post-CABG patient with segments of the heart that have not been adequately revascularized (Levitsky S. Protecting the myocardial cell during coronary revascularization. The William W. L. Glenn Lecture. Circulation 2006; 114(1 Suppl):I339-I343).

110. Ischemia/reperfusion involves transcription factors such as nuclear factor-kappa beta activation (NF-κB) and re-oxygenation is a key stimulus. Thus, NF-κB induces the rapid expression of several pro-inflammatory genes involved in ischemia-reperfusion, including TNFα, interleukins, chemokines and CAMs. Stimulation of cultured endothelial cells with pro-

inflammatory cytokines results in increased NF-κB activity, degradation of the inhibitory protein IκBα, and surface expression of VCAM-1, ICAM-1 and E-selectin while NO donors lead to decreased NF-κB activity, degradation of IκBα, and expression of VCAM-1, ICAM-1 and E-selectin. ROS stimulate PKC and activate NF-κB and this response is attenuated by NOdonors. It appears that NO acts as an antioxidant and inactivates ROS, increases levels of inhibitory proteins, and prevents the formation of substances that activate NF-κB and enhance the activity of proteins that inhibit NF-κB. Decreased NO after ischemia-reperfusion has been suggested to have the opposite effect, resulting in increased formation of proinflammatory enzymes, cytokines and adhesion molecules.

111. In general, small amounts of NO for short periods from eNOS are beneficial whereas large amounts of NO for sustained periods from iNOS are harmful. Some of the controversial effects of NO can be explained by differences in the severity and extent of the ischemia-reperfusion injury. Thus, large areas of severe injury, associated with very low flow and more prolonged ischemia, would be expected to induce more severe microvascular injury and inflammatory responses, greater release of ROS and cytokines, more peptides such as AngII, greater activation of iNOS, formation of more peroxynitrite and matrix metalloproteinases, and more cellular and structural disruption. This hypothesis is supported by the correlation found between expression of iNOS and eNOS genes with the degree of left ventricular dysfunction in dilated cardiomyopathy patients and the ifunctional regulation of apoptosis by NO. Since small amounts of NO are beneficial, especially during reperfusion after brief periods of ischemia as in ischemic preconditioning, therapies that enhance NO availability by increasing endothelial eNOS might be effective in recruiting left ventricular function in reperfused myocardium after coronary bypass surgery (Jugdutt BI. Nitric oxide and cardioprotection during ischemia-reperfusion. Heart Fail Rev 2002; 7:391-405).

112. In addition to the cardiac complications after coronary bypass surgery, neurological complications also occur as a result of I/R injury. In a prospective observational study of 2108 patients undergoing elective coronary artery bypass grafting (CABG) in 24 institutions in the USA, there was a 6% incidence of adverse cerebral outcomes. Patients were assessed for two types of neurological outcome: type I outcome, which included fatal or non-fatal stroke, stupor, or coma at discharge, occurred in 3%; and 3% of patients had type II outcomes that included deterioration in intellectual function, memory deficit, or seizures. In prospective studies of patients undergoing combined intracardiac and coronary artery surgery there was a 16% incidence of adverse cerebral outcomes, divided equally between type I (8%; 6% non-fatal strokes and 2% death from cerebral injury) and type II (7%) outcomes (Newman MF, Mathew JP, Grocott HP, Mackensen GB, Monk T, Welsh-Bohmer KA et al. Central nervous system injury associated with cardiac surgery. Lancet 2006; 368:694-703).

113. Whole body periodic acceleration, through the application of a motion platform, effectively achieves preconditioning prior to coronary bypass surgery. This was confirmed in a study of 20 anesthetized male swine weighing 40-50lb (Adams JA, Bassuk J, Wu H, Arias J, Wu D, Jarapur V et al. Preconditioning with periodic acceleration (pGz) prior to whole body ischemia reperfusion injury ameliorates myocardial stunning and arrhythmias. Circulation, abstract, in press. 2007). The pigs were placed on a motion platform and randomized to 1 hr of active pGz (3 Hz and Gz ± 0.4) (PC) or no activation for the same time

period, control (C). Ventricular fibrillation (VF) was electrically induced and unsupported for 8 min, followed by continuous manual chest compression and defibrillation until return of spontaneous circulation (ROSC) or a maximum period of 10 min. Echocardiograms to measure ejection fraction (EF%), fractional shortening (FS%) and wall motion score index (WMSI) were performed at baseline (BL), after pGz or control (BL2) and 30, 120 min after ROSC (ROSC30, ROSC120). All animals had ROSC after a median of 4 defibrillation attempts. PC animals had less hemodynamically significant arrhythmias in the first 30 mins ROSC; C (35) vs PRE (7) ($p< 0.05$) and less myocardial stunning as determined by echocardiography. Operation of the periodic acceleration according to the present invention causes release of NO from eNOS through increased pulsatile shear stress. Further, preconditioning with the periodic acceleration according to the present invention is non-invasive and drug-free. It is now clear from numerous peer-reviewed publications that various preconditioning methods or drugs have their basis in the early and later phases in activated eNOS. Furthermore, particularly in the late phase, an inflammatory response mediated by activation of NF-κß plays an important role in myocardial dysfunction.

114. Further, it appears that postconditioning is as effective as preconditioning in reducing myocardial infarct size and preserving endothelial function (Zhao, Z. Q., Corvera, J. S., Halkos, M. E., Kerendi, F., Wang, N. P., Guyton, R. A., and Vinten-Johansen, J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol, *285*: H579-H588, 2003). As pointed out by Zhao and associates, postconditioning may be clinically applicable in coronary interventions, coronary arterial bypass surgery, organ transplantation, and peripheral revascularization where reperfusion injury is expressed Adams and associates demonstrated that periodic acceleration produced postconditioning (Adams JA, Bassuk J, Wu H, Arias J, Wu D, Jarapur V et al. Post treatment with periodic acceleration (pGz) after cardiac arrest decreases acute post resuscitation myocardial stunning. Circulation, abstract, in press, 2007). Eighteen anesthetized male swine (40-50lbs) were studied. Ventricular fibrillation (VF) was electrically induced and unsupported for 8 min, followed by continuous manual chest compression and defibrillation until return of spontaneous circulation (ROSC) or 10 min. They were randomized to receive continuous pGz (frequency of 3 Hz and Gz ± 0.4) (POST CONDITIONING) for the remainder of the observation period or none, control (C). Echocardiograms to measure ejection fraction, fractional shortening and wall motion score index were performed at baseline (BL), and 30 and 120 min after ROSC (ROSC30, ROSC120). All animals had ROSC after a median of 4 defibrillation attempts. There were no differences between groups in defibrillation attempts, time to ROSC, arterial blood gases or hemodynamics over time. Compared to Controls, POST CONDITIONED animals had less acute myocardial stunning as evidenced by echocardiographic indices.

115. Pulsatile shear stress produced by the motion platform in addition to upregulating cardiac eNOS upregulates cardiac neuronal nitric oxide synthase (nNOS) (Wu H, Arias J, Bassuk J, Kurlansky P, Adams JA. Periodic acceleration (pGz) increases eNOS and nNOS in healthy rat heart. Circulation, abstract, in press. 2007). In whole body I/R after 18 minutes of CPR, intact basal nNOS activity is vital for survival from whole body ischemia reperfusion injury. iNOS inhibition as promoted by eNOS or pharmacologic agents prior to ischemia reperfusion, protects myocardial function after return of spontaneous circulation (ROSC) and decreases myocardial and brain hyperemic response after ROSC. Activated nNOS

is important to prevent cardiac arrhythmias in the reperfusion period, a complication of coronary bypass surgery (Adams JA, Wu D, Bassuk J, Arias J, Lozano H, Kurlansky P et al. Nitric oxide synthase isoform inhibition before whole body ischemia reperfusion in pigs: Vital or protective? Resuscitation 2007; in press; Wu H, Arias J, Bassuk J, Kurlansky P, Adams JA. Periodic acceleration (pGz) increases eNOS and nNOS in healthy rat heart. Circulation , abstract, in press, 2007).

116. **Basis for Utility of Claim 38.** The major complications of coronary bypass surgery relate to the consequences of ischemia/reperfusion injury. This affects all organs but is most life threatening and disabling to cardiac and central nervous system injury. The motion platform by causing pulsatile shear stress through whole body periodic acceleration releases nitric oxide into the circulation that promotes preconditioning and postconditioning thereby minimizing the effects of I/R injury associated with oxidative stress and inflammation. Further, activation of cardiac nNOS by pulsatile shear stress minimizes arrhythmias that occur in the reperfusion period.

Claim 39

117. Claim 39 recites "wherein periodic acceleration is used to treat and/or prevent obstructive sleep apnea syndrome commonly observed in patients with coronary artery disease". Obstructive sleep apnea (OSA) has been associated with cardiovascular complications that increase morbidity and mortality, including systemic hypertension, myocardial infarction, and cerebrovascular disease. Recent investigations, which established that OSA is an independent risk factor for these cardiovascular diseases, suggest that a specific causal relationship exists between the pathophysiology of OSA and cardiovascular dysfunction. However, mechanisms linking OSA with cardiovascular disease have not been fully identified. Several studies have demonstrated that OSA patients have increased plasma markers of systemic inflammation. In particular, increased levels of inflammatory cytokines, adhesion molecules, and activation of circulating neutrophils have been observed in patients with OSA. These studies suggest that activation of various inflammatory processes may directly contribute to atherogenesis and other cardiovascular diseases seen in OSA, and thus, these studies have opened a new direction of investigation into potential pathways linking the OSA with cardiovascular disease. Recurrent obstructive apneas induce repeated hypoxia and reoxygenation during sleep, which resembles the condition of ischemia-reperfusion, which has been shown to generate a large quantity of reactive oxygen species (ROS). While the degree of hypoxia occurring in association with OSA is less severe than in ischemia-reperfusion, cycles of hypoxia and reoxygenation often occur hundreds of times each night for many years. There is evidence for increased oxidative stress and ROS in OSA. The increased ROS generation resulting from recurrent hypoxia and reoxygenation may trigger the expression of multiple proinflammatory genes via activation of the oxidant-sensitive transcription factor nuclear factor κB (NF-κB). NF-κB is one of the most important redox responsive transcription factors, and it has been shown to play a crucial role in the activation of the promoter activity of over 200 genes, many of which play important roles in the pathophysiology of atherosclerosis and other cardiovascular diseases. Thus, OSA could promote systemic inflammation and cardiovascular pathology by inducing NF-κB-mediated expression of proinflammatory and proatherogenic genes.

118. Neutrophils in mild to moderate and severe OSA patients showed 4.8- and 7.9-fold greater NF-κB binding activity compared with control subjects. The degree of NF-κB activation was positively correlated with indices of apnea severity. In five severe OSA patients, 1 month of CPAP therapy decreased neutrophil NF-κB activation to control levels. sEselectin and sVCAM concentrations were reduced by CPAP in four of these five subjects. OSA leads to NF-κB activation, which constitutes an important pathway linking OSA with systemic inflammation and cardiovascular disease (Htoo AK, Greenberg H, Tongia S, Chen G, Henderson T, Wilson D et al. Activation of nuclear factor kappaB in obstructive sleep apnea: a pathway leading to systemic inflammation. Sleep Breath 2006; 10:43-50).

119. **Basis for Utility of Claim 39.** Obstructive sleep apnea is associated with oxidative stress and inflammation and is a form of ischemia/reperfusion injury. This is a risk factor for coronary artery disease. The motion platform by causing pulsatile shear stress through whole body periodic acceleration releases nitric oxide into the circulation that promotes preconditioning and postconditioning thereby minimizing the effects of I/R injury associated with oxidative stress and inflammation.

Claim 40

120. Claim 40 recites "wherein periodic acceleration is used to treat and/or prevent cognitive deficits, learning deficits, and/or behavioral abnormalities in early cognitive impairment". It is well known that aging leads to a progressive loss of cognitive function, and there is abundant evidence that age-associated learning and memory impairments are related to decreased function in the medial temporal lobe and frontal cortex. It has been proposed that nitric oxide (NO) plays an important role in the aging process. Aged rats (24 months old) showed reduced exploratory activity and poorer spatial learning relative to the young adults (4 months old). Significant increases in total NOS activity were found in the aged dentate gyrus and a dramatic decrease in eNOS expression was observed in the aged CA2/3. A strong positive correlation between CA1 eNOS protein expression and swimming speed in the water maze task reflects a relationship between the local cerebral blood flow and neuronal activity. (Liu P, Smith PF, Appleton I, Darlington CL, Bilkey DK. Hippocampal nitric oxide synthase and arginase and age-associated behavioral deficits. Hippocampus 2005; 15:642-655).

121. Nitric oxide (NO) contributes widely to synaptic plasticity in the CNS. According with the presumed significance of this phenomenon, NO also participates in several types of learning behavior. NO release in the brain is typically linked to activation of NMDA receptors to which the Ca2_/calmodulin-dependent neuronal NO synthase isoform (nNOS) is tethered and which trigger long-term potentiation (LTP) and other types of synaptic plasticity. On applying LTP-inducing stimuli, NMDA receptor activation elicits a brief burst of NO. In this scenario, NO from nNOS is a good candidate for a retrograde messenger, diffusing rapidly to the presynaptic terminal and effecting changes in the neurotransmitter release machine. Postsynaptic effects of NO have also been found. Despite the attractiveness of nNOS-derived NO as a messenger associated with NMDAreceptor activation and LTP, NO-dependent LTP was found to be preserved in nNOS-deficient mice but lost when eNOS was knocked out.

122. Administration of exogenous NO paired with a weak titanic stimulation of afferent fibers generates an NMDA receptor in dependent LTP, a result that fulfils a key prediction of the retrograde messenger hypothesis. However, for exogenous NO to potentiate synaptic transmission in this way, a source of endogenous NO is needed, both before and after the pairing protocol. Studies on LTP itself also emphasized the importance of tonic NO in that block of NO synthesis shortly (5 min) after tetanizing the afferent pathway inhibited LTP whereas doing so later (15 min post-tetanus) was ineffective. Collectively, these results imply that tonic and phasic NO signals may both be involved.

123. It appears that eNOS in endothelial cells generates the tonic level of NO whereas nNOS present in hippocampal pyramidal cells produces the phasic NO signal in association with NMDA receptor stimulation and that both are important for CA1 hippocampal LTP. Expanding on the finding that blood vessels signal to central axons through NO, the notion emerges that blood vessels can influence synaptic plasticity in the brain, also through release of NO. Circumstantial evidence consistent with this hypothesis derives from behavioral tests in several species, indicating that tonic NO and cGMP contribute to memory formation. Furthermore, as in the hippocampus, defective synaptic plasticity has been recorded in eNOS knock-outs in the cerebral cortex and striatum, where eNOS is also localized to blood vessels. In the solitary tract nucleus, eNOS regulates autonomic function and mice lacking eNOS exhibit various behavioral and neurochemical abnormalities, and decreased neurogenesis. Therefore, the line of communication between endothelial cells and cerebral neurons might be widespread (Hopper RA, Garthwaite J. Tonic and phasic nitric oxide signals in hippocampal long-term potentiation. J Neurosci 2006; 26:11513-11521).

124. An aging rat model of chronic brain hypoperfusion (CBH) that mimics human mild cognitive impairment (MCI) has been used to examine the role of nitric oxide synthase (NOS) isoforms on spatial memory function. Rats with CBH underwent bilateral common carotid artery occlusion (2-vessel occlusion (2-VO)) for either 26 or 8 weeks and were compared with nonoccluded sham controls (S-VO). The neuronal and endothelial (nNOS/eNOS) constitutive inhibitor nitro-L-arginine methyl ester (L-NAME) 20 mg/kg was administered after 26 weeks for 3 days to 2-VO and S-VO groups and spatial memory was assessed with a modified Morris watermaze test. Only 2-VO rats worsened their spatial memory ability after L-NAME. Electron microscopic immunocytochemical examination using an antibody against eNOS showed 2-VO rats had significant loss or absence of eNOS-containing positive gold particles in hippocampal endothelium and these changes were associated with endothelial cell compression, mitochondrial damage and heavy amyloid deposition in hippocampal capillaries and perivascular region. In the 8-week study, three groups of 2-VO rats were administered an acute dose of 7-NI, aminoguanidine or L-NIO, the relatively selective inhibitors of nNOS, inducible NOS and eNOS. Only rats administered the eNOS inhibitor L-NIO worsened markedly their watermaze performance (P¼0.009) when compared with S-VO non-occluded controls. Therefore, vascular nitric oxide derived from eNOS may play a critical role in spatial memory function during CBH possibly by keeping cerebral perfusion optimal through its regulation of microvessel tone and cerebral blood flow and that disruption of this mechanism can result in spatial memory impairment.

125. There is a dose dependent effect of lead intoxication in adult rats on spatial memory, without affecting spatial learning. A similar cognitive impairment is also evident in the passive avoidance task, but not all these impairments are related to motor activity problems since the cue maze was not affected. Hippocampal LTP is also affected in a dose-dependent manner. The decrease activity of cNOS can be playing an important role in the cognitive impairments observed after sub-chronic consumption of lead, along with other possible molecular mechanism related with synaptic plasticity. However, this molecular deficit seems to be particularly robust in regions such as the hippocampus and cerebellum (Garcia-Arenas G, Ramirez-Amaya V, Balderas I, Sandoval J, Escobar ML, Rios C et al. Cognitive deficits in adult rats by lead intoxication are related with regional specific inhibition of cNOS. Behav Brain Res 2004; 149:49-59).

126. Whole body periodic acceleration produces a significant increase of blood flow as determined with colored microspheres to the cerebrum (183%) and brain stem (177%) compared to the control state in anesthetized pigs (Adams JA, Mangino MJ, Bassuk J, Kurlansky P, Sackner MA. Regional blood flow during periodic acceleration. Crit Care Med 2001; 29:1983-1988).

127. Therefore, through activation of eNOS, the motion platform has tissue and animal support for treating and/or preventing cognitive deficits, learning deficits, and/or behavioral abnormalities in early cognitive impairment. Activation of eNOS from the vascular side enhances tonic regulation of Long-Term Potentiation in the hippocampus, region responsible for memory and learning. Increased eNOS activity also produces a significant global increase in brain blood flow that also aids memory and cognition. With hypoperfusion and lead intoxication, memory and learning are impaired.

128. **Basis for Utility of Claim 40.** Treatment with the motion platform has excellent tissue and animal support for this claim of treating and/or preventing cognitive deficits, learning deficits, and/or behavioral abnormalities in early cognitive impairment. Activation of eNOS from the vascular side enhances tonic regulation of Long-Term Potentiation (the basis of memory) in the hippocampus, the region responsible for memory and learning. Increased eNOS activity also produces a significant global increase in brain blood flow that also aids memory and cognition. With hypoperfusion and lead intoxication, memory and learning are impaired.

Claim 41

129. Claim 41 is amended to recite "wherein periodic acceleration is used to treat and/or prevent Alzheimer's disease, vascular dementias, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's chorea, and Wilson's disease". These chronic neurological diseases have as their basis increased oxidative stress and chronic inflammation marked by increased activity of nuclear factor kappa beta. These factors selectively destroy neurologic tissues giving rise to characteristic symptoms (Mattson MP, Camandola S. NF-kappaB in neuronal plasticity and neurodegenerative disorders. J Clin Invest 2001; 107:247-254; Napoli C, Palinski W. Neurodegenerative diseases: insights into pathogenic mechanisms from atherosclerosis. Neurobiol Aging 2005; 26:293-302).

130. The motion platform produces pulsatile shear stress that causes release of nitric oxide into the circulation from activation of eNOS. NO diminishes oxidative stress and inhibits nuclear factor kappa beta thereby serving as treatment and prevention in these neurological diseases.

131. **Basis for Utility of Claim 41.** The motion platform produces pulsatile shear stress that causes release of nitric oxide into the circulation from activation of eNOS. NO diminishes oxidative stress and inhibits nuclear factor kappa beta thereby serving as treatment and prevention in these neurological diseases.

Claim 42

132. Claim 42 recites "wherein periodic acceleration is used to treat and/or prevent cardiac allograft vasculopathy". The prevalence of occlusive coronary artery disease of the cardiac allograft (cardiac allograft vasculopathy [CAV]) is 10% to 15% at 1 year and 40% to 50% at 5 years after transplantation. The leading cause of death in patients who survive beyond the first year after heart transplantation, CAV frequently presents with sudden death, myocardial infarction, and decline in cardiac function. Retransplantation, the only effective therapy for established disease, is seriously limited by inferior survival rates of 60% at 1 year and 30% at 5 years, compared with survival results of first allografts of 85% and 60%, respectively. CAV may be viewed within a pathophysiologic context in which endothelial injury plays a central role. Endothelial cells play a crucial role in maintaining normal vessel wall function by their ability to inhibit thrombus formation, leukocyte adhesion, and vascular smooth muscle cell proliferation, and by regulation of vessel tone. Coronary vessels of the transplanted heart are exposed to multiple environments that induce endothelial cell injury, including brain death, ischemia-reperfusion, alloimmune responses, viral infections, and the metabolic abnormalities that frequently develop as a consequence of the immunosuppressive drugs used in heart transplant recipients. Thus, endothelial cell injury is capable of initiating local vascular events that are known to be important early triggers of atherosclerosis. The evidence indicates that an important common pathway is a derangement in the eNOS system leading to NO deficiency within the allograft. Prevention strategies that target individual components of the pathways leading to endothelial dysfunction, such as hyperlipidemia and inhibition of cellular proliferative responses have been utilized. However, strategies that directly restore the eNOS pathway offer potential for a more complete inhibition of CAV than has been achieved.

133. The pathogenesis of cardiac allograft vasculopathy (CAV), a major cause of death in patients surviving more than 1 year after heart transplantation, involves several immunologic and metabolic factors, including ischemia/reperfusion injury, human leukocyte antigen mismatch, viral infection, hyperlipemia, and hypertension. In addition to these well-known risk factors, markers of the Metabolic Syndrome are associated with increased incidence of CAV and poor prognosis after heart transplantation. Systemic inflammation is believed to represent an important mechanistic pathway through which metabolic risk factors may lead to vascular disease. Although inflammation is regulated by a complex interaction between systemic mediators and locally acting cytokines and chemokines, C-reactive protein (CRP) has been widely chosen as a single, easily assessable marker that may recapitulate the level of systemic inflammation in the setting of a daily clinical practice. Indeed, CRP has been well

characterized as a relevant risk factor for atherosclerosis and cardiovascular events in the general population. Although a few studies have suggested that CRP is similarly associated with increased risk of them have specifically investigated the relationship between systemic inflammation and metabolic impairment in heart transplant recipients. An active interplay between systemic inflammation and markers of metabolic dysregulation increases the risk for CAV and subsequently worsen cardiovascular prognosis. In heart transplant recipients, systemic inflammation may be an important mediator of graft vascular injury associated with metabolic syndrome (Valantine HA. Cardiac allograft vasculopathy: central role of endothelial injury leading to transplant "atheroma." Transplantation 2003; 76:891-899; Biadi O, Potena L, Fearon WF, Luikart HI, Yeung A, Ferrara R et al. Interplay between systemic inflammation and markers of insulin resistance in cardiovascular prognosis after heart transplantation. J Heart Lung Transplant 2007; 26:324-330).

134. C-reactive protein (CRP) is an acute-phase reactant that activates complement. Its hepatic synthesis is stimulated by interleukin-6 to yield levels that can rise 500-fold within 24 to 48 hours of the initiation of an inflammatory process. In addition to participating in immune response, CRP has received considerable attention as a risk factor for cardiovascular disease, with chronic modest elevations being associated with a greater likelihood of myocardial infarction, sudden cardiac death, stroke, and peripheral vascular disease. CRP is also a risk factor for the progression of subclinical vascular disease and for hypertension. In vitro studies have shown that CRP decreases the expression of endothelial NO synthase (eNOS). In addition to a variety of other actions important to cardiovascular health, endothelium-derived NO promotes endothelial cell (EC) growth and migration and angiogenesis, which underlie both neovascularization and the maintenance of intimal layer integrity. An intact endothelial monolayer modulates hemostasis and thrombolysis and provides a nonpermeable barrier protecting vascular smooth muscle cells from circulating growth factors. Disruption of the intimal layer, either by gross denudation related to a vascular intervention or gap formation between ECs caused by disturbed shear stress, places the arterial wall at greater risk for vascular disease. CRP downregulates eNOS and attenuates reendothelialization in vivo in mice (Schwartz R, Osborne-Lawrence S, Hahner L, Gibson LL, Gormley AK, Vongpatanasin W et al. C-reactive protein downregulates endothelial NO synthase and attenuates reendothelialization in vivo in mice. Circ Res 2007; 100:1452-1459).

135. Thus, CAV is due to non-specific systemic inflammation although its prevalence is especially high in the Metabolic Syndrome. CAV is manifested by endothelial dysfunction with nitric oxide deficiency. CRP is a marker of inflammation but also downregulates activity of eNOS. Therefore, treatment of CAV must be directed to upregulating eNOS activity. This can be accomplished by increasing pulsatile shear stress with the motion platform to upregulate eNOS (Wu H, Arias J, Bassuk J, Kurlansky P, Adams JA. Periodic acceleration (pGz) increases eNOS and nNOS in healthy rat heart. Circulation , abstract, in press. 2007).

136. **Basis for Utility of Claim 42.** Cardiac allograft vasculopathy (CAV) is a life threatening condition in transplant patients due to the presence of systemic inflammation that causes endothelial dysfunction, downregulation of eNOS and nitric oxide deficiency.

Upregulation of eNOS with the motion platform restores eNOS activity and promotes nitric oxide release into the circulation thus antagonizing the adverse effects of this condition.

Claim 43

137. Claim 43 recites "wherein periodic acceleration is used to promote angiogenesis in ischemic tissues". NO plays a critical role in ischemia-induced angiogenesis. Impairment of this metabolic pathway is relevant in the development of peripheral arterial occlusive disease. Studies show that (i) NO levels are increased in the ischemic limb; (ii) pharmacological inhibition or gene disruption of eNOS decreases NO levels and inhibits ischemia-induced angiogenesis; (iii) supplementation of NO, by the use of exogenous sources, restores ischemia induced angiogenesis; and (iv) cardiovascular diseases associated with decreased NO synthesis have impaired ischemia-induced angiogenesis. Agonist-dependent NO release restores ischemia-induced angiogenesis in these pathological situations. Therefore, increased local concentrations of NO should be sufficient to stimulate angiogenesis. The restoration of normal NO levels in diseased arteries is thus a major therapeutic goal which could be achieved by supplementation with exogenous NO. However, NO has systemic effects that represent an obstacle to this goal (Contreras DL, Robles HV, Romo E, Rios A, Escalante B. The role of nitric oxide in the post-ischemic revascularization process. Pharmacol Ther 2006; 112:533-563). Whole body periodic acceleration to produce nitric oxide does not have adverse systemic effects like exogenous NO donor drugs and thus is the optimal therapeutic strategy to promote angiogenesis in ischemic tissues.

138. **Basis for Utility of Claim 43.** The utility of this claim is based upon sound evidence that nitric oxide deficiency is present in ischemic tissues and that adequate quantities are necessary for angiogenesis. Whole body periodic acceleration that promotes NO release into the circulation corrects nitric oxide deficiency.

Claim 44

139. Claim 44 recites "wherein periodic acceleration is used to manage hereditary hemorrhagic telangiectasia". Mutations in the endoglin (ENG) gene cause hereditary hemorrhagic telangiectasia type 1 (HHT1), also known as Rendu–Osler–Weber syndrome. HHT is an autosomal dominant vascular dysplasia that affects 1:10 000 individuals. This disorder is associated with nasal bleeding and overexpression of networks of fine vessels in the skin in the majority of patients and with pulmonary and cerebral arteriovenous malformations that are more frequent, particularly in HHT1 patients. Such malformations may rupture leading to massive hemorrhage, stroke or death. ENG mutations are distributed throughout the gene and lead to haploinsufficiency, indicating that endoglin levels are critical in maintaining vascular homeostasis. The vascular endothelium secretes vasodilators including NO, which is produced mostly by endothelial NO synthase (eNOS), and prostacyclin (PGI2) and prostaglandin E2 (PGE2), which are produced by cyclooxygenases (COXs). Endoglin is a regulatory component of the TGF-ß receptor system in endothelial cells capable of modulating specific responses to this multipotent growth factor. Its reduced expression may result in disruption of the delicate balance in the secretion of endothelium-derived vasodilators and vasoconstrictors, thus inducing changes in vascular tone regulation. In a mice model of HTT, there is impaired eNOS activity

due to uncoupled eNOS. COX-2 is elevated suggesting that endoglin plays a role in the maintenance of vascular homeostasis and the fine balance between eNOS and COX-2 in endothelial cells. Thus, there is upregulation of endothelial COX-2 and a consequent increase in vasodilator prostaglandin production in a mouse model of HHT1. The inverse correlation between endoglin and COX-2 expression occurs in endothelial and nonendothelial cells and indicates that endoglin modulates COX-2 expression. Further, the deficiency in nitric oxide and increase in superoxide from eNOS uncoupling account for the rise in COX-2. It is this imbalance in vasculat mediators that produces the pathology in HTT1 (Jerkic M, Rivas-Elena JV, Santibanez JF, Prieto M, Rodriguez-Barbero A, Perez-Barriocanal F et al. Endoglin regulates cyclooxygenase-2 expression and activity. Circ Res 2006; 99:248-256; Toporsian M, Gros R, Kabir MG, Vera S, Govindaraju K, Eidelman DH et al. A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. Circ Res 2005; 96:684-692).

140. Both the deficiency of nitric oxide and the eNOS uncoupling can be reversed by the increased shear stress associated with application of whole body periodic acceleration (Lam CF, Peterson TE, Richardson DM, Croatt AJ, d'Uscio LV, Nath KA et al. Increased blood flow causes coordinated upregulation of arterial eNOS and biosynthesis of tetrahydrobiopterin. Am J Physiol Heart Circ Physiol 2006; 290:H786-H793).

141. **Basis for Utility of Claim 44.** The utility of this claim is based upon sound evidence that nitric oxide deficiency and uncoupled eNOS are present in hereditary hemorrhagic telangiectasia that adequate quantities of nitric oxide from coupled eNOS are necessary to reverse this abnormality. Whole body periodic acceleration that promotes NO release into the circulation corrects nitric oxide deficiency and induces change from the uncoupled to the normal coupled form of eNOS.

Claim 45

142. Claim 45 recites "wherein periodic acceleration is used to treat and/or prevent migraine". Migraine, a chronic disease afflicting a substantial proportion of the population, is generally characterized by severe, throbbing headache accompanied by nausea and vomiting with enhanced sensitivity to light, sound, and smells. The intracranial throbbing pain of migraine is mediated primarily by neuronal activity along the trigeminovascular pathway. Activation and sensitization of primary afferent nociceptive (sensory pain receptor) neurons that innervate the intracranial meninges and their related blood vessels (ie, meningeal nociceptors) is the first step in driving this sensory nociceptive system to promote the sensation of pain. While the exact mechanisms underlying activation and sensitization of meningeal nociceptors are not completely understood, local inflammation and release of mediators are thought to play a key role. Triggering mechanisms underlying these immune and neuronal responses are currently unknown. Migraine attacks are triggered by a variety of conditions. Hypothesized endogenous triggers include abnormal cortical activity reflected in the phenomenon of cortical spreading depression, which is a slowly propagating ionic disturbance within the cerebral cortex that has been implicated in the migraine aura, hyperexcitability of the parasympathetic system, release of hormones involved in the stress response, and in female patients changes in the level of sex

hormones associated with the menstrual cycle. Exogenous triggers may include dietary elements such as cheese, chocolate, coffee, wine, and changes in weather patterns.

143. The notion that all of these seemingly unrelated factors are linked to the emergence of the episodic intracranial head pain of migraine raises the possibility of a single biological phenomenon that is impinged upon by these various triggers and that plays a critical role in promoting meningeal inflammation and the ensuing activation and sensitization of meningeal nociceptors. The emergence of a local inflammatory response in the meninges is a potential contributor to the activation and sensitization of meningeal nociceptors during migraine. Activation of resident immune cells such as macrophages and mast cells, which are a prominent feature of the intracranial meninges, is likely to serve as a critical step in promoting the enhanced excitability of meningeal nociceptors. Meningeal mast cells are of special interest by virtue of their proximity to meningeal blood vessels and pain fibers, their ability to release a host of pronociceptive mediators, and their propensity to be activated by the various migraine triggering phenomena.

144. Mast cells are ubiquitous immunocompetent cells that participate in numerous physiological and pathophysiological conditions. Mast cells originate in the bone marrow and migrate into connective tissues and mucosal surfaces, where they gain mature morphologic and functional characteristics under the influence of local microenvironmental factors. While mast cells share many characteristics such as their prototypical cytoplasmic granules, they differ in characteristics including size, granule contents, and receptor expression. Although mostly known for their role in allergic responses, mast cells are important cellular components that mediate other immune responses. Activation of mast cells results in the release of granule associated mediators (ie, degranulation) including histamine, serotonin, nerve growth factor, proinflammatory cytokines such as TNF-α, IL-1, and IL-6, and various proteases such as tryptase. In various activation modes, mast cells can also generate *de novo* lipid-derived mediators, including leukotrienes and prostanoids, and can synthesize nitric oxide from iNOS. The primary cause of mast cell activation has been considered to be the cross-linking of the high affinity IgE receptor (FcεRI) by IgE and multivalent antigen—a process that culminates in the explosive release of preformed mediators (ie, immunological activation) to produce the typical anaphylactic allergic responses. However, non-antigenic pathways of activation have also been implicated in many pathologic conditions. For example, free immunoglobulin, hormones, various cytokines, and vanilloid compounds are known to promote mediator secretion from mast cells. Polycationic compounds such as the neuropeptide substance P and bradykinin promote mast cell activation and degranulation. The evidence for mast cell degranulation as a factor in the pathophysiology of migraine headache is circumstantial but a potential contributing cause (Levy D, Burstein R, Strassman AM. Mast cell involvement in the pathophysiology of migraine headache: A hypothesis. Headache 2006; 46 Suppl 1:S13-S18). Mast cell degranulation with antigen challenge is minimized by application of whole body periodic acceleration due to release of nitric oxide into the circulation (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752).

145. Although some investigators have postulated that migraine headaches arise from vasodilation of cranial blood vessels, glyceryl trinitrate (GTN) provokes typical

migraine headaches 4 to 6 hours after its infusion in two-thirds of migraineurs, a period long after its vasodilatory effects have dissipated. The administration of an NOS inhibitor significantly improves symptoms in two-thirds of subjects during an acute attack. Upregulation of genes relevant to pathogenesis of an attack might explain the latency of 4 to 6 hours for migraine headache following GTN infusion. It has been found using a GTN dose relevant to humans that inducible NOS (iNOS) induction within dura mater, an important pain-sensitive intracranial tissue, occurs 4 to 6 hours after GTN infusion and not earlier. Furthermore, iNOS expression is predominantly found within macrophages. Edema and mast cell degranulation, considered fundamental to inflammation and migraine pathogenesis, are detected with a similar time course; in addition, levels of the cytokines interleukin (IL)-1ß and IL-6 increased. The transcription factor nuclear factor–kappa beta (NF-κB) plays a pivotal role in iNOS induction and controls transcription of acute phase proteins, including cytokines, adhesion molecules, and antioxidant enzymes, among others. Parthenolide, an abundant sesquiterpene lactone found in the medical herb feverfew *(Tanacetum parthenum)*, has been used successfully in the treatment of inflammatory conditions and migraine. In two of the three largest placebo-controlled, double-blind studies of feverfew, prophylactic treatment with the herb reduced migraine attacks and pain intensity. The mechanism of action for the anti-migraine efficacy of feverfew is not known. However, recent reports suggest that parthenolide targets the NF-κB complex to block transcription of inflammatory proteins. Because iNOS contributes to delayed meningeal inflammation, the regulation of iNOS gene expression by NF-κB in rat dura mater was investigated and correlated to the delayed meningeal events that develop following delivery of NO donors.

146. Targeting the inflammatory response by selectively inhibiting NF-κB driven transcription downstream for pro-inflammatory gene expression and iNOS activity has potential as an effective therapeutic approach to treatment of headache. Indeed, GTN infusion causes a prototypical induction of migraine headache in susceptible humans, and the cellular and molecular features of this response in rodents resemble what has been found in other experimental migraine models, ie, delayed plasma protein extravasation, mast cell degranulation, and cytokine release. In addition, GTN increases electrophysiologically recorded neuronal responses to facial cutaneous stimuli within the trigeminal nucleus caudalis and increases early immediate gene response within this nucleus (Reuter U, Chiarugi A, Bolay H, Moskowitz MA. Nuclear factor-kappa B as a molecular target for migraine therapy. Ann Neurol 2002; 51:507-516).

147. Nitric oxide released from eNOS by pulsatile shear stress when whole body periodic acceleration is applied inhibits NF-κB activity (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752; Blais V, Rivest S. Inhibitory action of nitric oxide on circulating tumor necrosis factor-induced NF-kappaB activity and COX-2 transcription in the endothelium of the brain capillaries. J Neuropathol Exp Neurol 2001; 60:893-905; Colasanti M, Persichini T. Nitric oxide: an inhibitor of NF-kappaB/Rel system in glial cells. Brain Res Bull 2000; 52:155-161). Inhibition of NF-κB and iNOS activity as well as prevention of degranulation of mast cells all take place with nitric oxide released from eNOS with whole body periodic acceleration.

148. **Basis for Utility of Claim 45.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration prevents and treats the immunologic factors associated with migraine headaches, e.g., increased NF-κB and iNOS activity as well as degranulation of mast cells.

Claim 46

149. Claim 46 recites "wherein periodic acceleration is used to treat the inflammation attendant with prion diseases". Prion disorders are a group of transmissible diseases with common pathological changes of the central nervous system (CNS), including neuronal cell loss, vacuolation, astrocytosis, and, frequently, the presence of amyloid plaques. Creutzfeldt-Jakob disease (CJD) in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) or mad cow disease in cows are the most extensively studied prion diseases. Oxidative stress is induced by reactive oxygen species (ROS) or free radicals, and plays an important role in the pathogenesis of several neurodegenerative disorders including Alzheimer's disease. In addition to the well-known toxic nature of ROS in these diseases, low concentrations of these compounds cause small perturbations in the cellular redox state and may function as intracellular effectors of gene transcription. NF-κB is known to be a major transcriptional activator for inflammatory mediators in a variety of cells, including those in the brain. As a model for prion diseases, scrapie in sheep has been studied. Proinflammatory cytokine genes such as interleukin-1α/β and TNF-α were expressed only in the brains of the scrapie-infected group compared to a control group. Immunoreactivity of NF-κB was localized in astrocytes and prion protein is known to accumulate in astrocytes prior to the cardinal neuropathological changes in scrapie. Therefore, the increase in NF-κB activity might be the result of the accumulation of prion protein in astrocytes via increased ROS formation. This concept is supported further the finding that there was induction of two representative target genes of NF-κB activation, interleukin-6 and iNOS, in brain astrocytes of scrapie-infected animals. Therefore, prion infection in brain induces oxidative stress and changes in iron metabolism. This increase in oxidative stress directly damages neuronal cells and indirectly affects the signaling function in glial cells, resulting in indirect neuronal cell loss through the production of cytotoxic mediators (Kim JI, Choi SI, Kim NH, Jin JK, Choi EK, Carp RI et al. Oxidative stress and neurodegeneration in prion diseases. Ann N Y Acad Sci 2001; 928:182-186).

150. Whole body periodic acceleration through the release of nitric oxide from eNOS by pulsatile shear stress treats the inflammation attendant with prion diseases. The basis for these benefits is accomplishes this treatment by inhibition of NF-κB, iNOS and TNF-α activities (Abraham WM, Ahmed A, Serebriakov I, Lauredo IT, Bassuk J, Adams JA et al. Whole-body periodic acceleration modifies experimental asthma in sheep. Am J Respir Crit Care Med 2006; 174:743-752; Blais V, Rivest S. Inhibitory action of nitric oxide on circulating tumor necrosis factor-induced NF-kappaB activity and COX-2 transcription in the endothelium of the brain capillaries. J Neuropathol Exp Neurol 2001; 60:893-905; Colasanti M, Persichini T. Nitric oxide: an inhibitor of NF-kappaB/Rel system in glial cells. Brain Res Bull 2000; 52:155-161). Further, nitric oxide released from eNOS scavenges ROS present in prion diseases).

151. **Basis for Utility of Claim 46.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration inhibits inflammatory mediators present in prion diseases such as NF-κB, IL-1α and IL-1B as well as scavenging ROS.

Claim 47

152. Claim 47 recites "wherein periodic acceleration is used to manage the aging process". Aging is a multifactorial process with factors that include 1) increased oxidative stress, 2) increased inflammation, 3) overnutrition, and 4) shortening of telomeres with each round of DNA replication. No single factor completely explains the aging process. Nitric oxide as released from eNOS with whole body periodic acceleration through its antioxidant and anti-inflammatory actions minimizes the deleterious effects of these factors thereby managing the aging process.

153. Oxidative stress. The aging process is the accumulation of oxidative damage to cells and tissues, associated with a progressive increase in the chance of morbidity and mortality. During lifetime, an antioxidant network counteracts the deleterious action of free radicals and reactive species on macromolecules. Cells synthesize some of their antioxidants, as the enzymes superoxide dismutase, catalase, and glutathione peroxidase, as well as the peptides with thiol groups, as glutathione and nitric oxide from eNOS. Other antioxidants are obtained through nutrition, as vitamin C, vitamin E, and carotenoids. Longliving animal species have more efficient antioxidant systems and higher liver Cu, Zn superoxide dismutase activity than shorter-living species. Several repair systems help antioxidant action by the recovery of damaged macromolecules. Together, these systems play an important role in the ability of the body to respond to the oxidant challenge of using molecular oxygen to drive reactions that yield the necessary energy for life processes. Oxidative stress is classically defined as a redox unbalance with an excess of oxidants or a defect in antioxidants (Junqueira VB, Barros SB, Chan SS, Rodrigues L, Giavarotti L, Abud RL et al. Aging and oxidative stress. Mol Aspects Med 2004; 25:5-16).

154. Mitochondria in mammalian cells are the major energy suppliers that generate ATP through oxidative phosphorylation. The mitochondrial respiratory chain is also a major intracellular source of reactive oxygen species (ROS) and free radicals under normal physiologic and pathologic conditions. It has been postulated that loss of mitochondrial function and increased mitochondrial ROS production are important causal factors in aging. Accumulation of somatic mutations in mtDNA is a major contributor to human aging and degenerative diseases. As a result of this new wave of mitochondrial research, the free radical theory of aging (increased oxidative stress) has thus been extended to the "mitochondrial theory of aging". The mitochondrial theory of aging proposes that progressive accumulation of somatic mutations in mtDNA during an individual's lifetime leads to a decline in the bioenergetic function of mitochondria and is a contributory factor to human aging. ROS are generated at very low levels during mitochondrial respiration under normal physiologic conditions. Oxidative damage to mtDNA by ROS may lead to DNA strand breaks and the occurrence of somatic mtDNA mutations. Accumulation of these mtDNA mutations may result in dysfunction of the respiratory chain, leading to increased ROS production in mitochondria and subsequent

accumulation of more mtDNA mutations. This vicious cycle has been proposed to account for an increase in oxidative damage during aging, which leads to the progressive decline of cellular and tissue functions as a result of an insufficient supply of energy and/or increased susceptibility to apoptosis.

155. Most studies conducted on cultured human cells and animals have revealed that aging is associated with impairment of bioenergetic functions, increased oxidative stress, attenuated ability to respond to stresses, and increased risk of contracting cancers and age-associated diseases. These characteristics and phenomena gradually occur in advanced age in organs and tissue cells, which are usually correlated with mitochondrial ROS production, oxidative damage, accumulation of mtDNA mutations, mitochondrial dysfunction, activation of apoptosis/necrosis, and altered expression of specific clusters of genes. During aging the expression levels of the genes that increase normally in response to DNA damage and oxidative stress are increased, whereas those involved in energy metabolism, biosynthesis, and protein turnover are decreased. Caloric restriction can reverse or retard these changes in the gene expression during aging, extend life span, slow down aging associated physiologic changes, and reduce cancer incidence in rodents and primates. These findings suggest that modulating the rate of energy metabolism may bring about changes in the redox status, mitochondrial function, and genomic integrity of animal cells (Lee HC, Wei YH. Oxidative stress, mitochondrial DNA mutation, and apoptosis in aging. Exp Biol Med (Maywood) 2007; 232:592-606).

156. Researchers have generally equated ROS production to the mitochondrial respiratory chain without even discussing the alternatives. Indeed, when the ROS theory of aging was developed, the NOX family of NADPH oxidases was not known, nor was its existence even suspected. Thus, NADPH oxidases are the new kids in the block and simply not sufficiently well known to many aging researchers. Aging through ROS generation by the mitochondrial respiratory chain fits well with earlier theories suggesting an inverse relationship between the metabolic rate and the longevity of an organism. However, in reality there is not a good correlation between the metabolic rate of different organisms and their longevity. Also, respiratory rate often does not correlate with ROS levels and faster respiration may be correlated with lower, not higher generation of ROS. Oxidative damage can be observed in mitochondria during aging and mitochondrially targeted ROS scavengers may slow down aging. However, while oxidative damage to mitochondria is undisputed, this does not provide a strong argument in favor of ROS generation by the mitochondrial respiratory chain, as the site of oxidative damage is not necessarily the site of ROS generation. To make things more complicated, there are increasingly reports suggesting that at least some NOX isoforms might localize to mitochondria. Thus, even ROS generation within mitochondria should not be invariably attributed to the mitochondrial respiratory chain. mtNOS cannot account for the vast majority of cellular ROS. Indeed, many studies suggesting that mitochondria produce high levels of ROS were performed using isolated (thus potentially damaged) mitochondria. Detectable mitochondrial ROS generation in intact cells typically requires exceedingly high glucose concentrations. Thus, activation of NADPH oxidases appears an important source of ROS as another factor in the oxidative stress of aging (Krause KH. Aging: a revisited theory based on free radicals generated by NOX family NADPH oxidases. Exp Gerontol 2007; 42:256-262).

157. Age-related cardiac vulnerability and vascular changes are accompanied by increased superoxide production. This increase in superoxide production, particularly at the vascular level, is partly due to the increased expression of NADPH oxidases and partly due to uncoupling of eNOS (Oudot A, Martin C, Busseuil D, Vergely C, Demaison L, Rochette L. NADPH oxidases are in part responsible for increased cardiovascular superoxide production during aging. Free Radic Biol Med 2006; 40:2214-2222). In healthy humans, impaired endothelial function as indicated with reduced endothelium dependent dialation of arterial blood flow with aging is associated with increased expression of NAD(P)H that contributes to oxidative stress (Donato AJ, Eskurza I, Silver AE, Levy AS, Pierce GL, Gates PE et al. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. Circ Res 2007; 100:1659-1666).

158. Diminished NO bioavailability by increased superoxide production is one of the major mechanisms responsible for the impaired endothelium-dependent vasodilator responses in hypertension and aging. Increased production of superoxide, reduced activity of superoxide dismutase, and impaired shear stress-induced activation of eNOS are the causes of decreased shear stress-induced release of NO in blood vessels of aged rats (Sun D, Huang A, Yan EH, Wu Z, Yan C, Kaminski PM et al. Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. Am J Physiol Heart Circ Physiol 2004; 286:H2249-H2256). In experimental and human essential hypertension, activation of NADPH oxidase and xanthine oxidase are the two major sources responsible for the increased superoxide production. Vascular superoxide can be also produced by eNOS uncoupling, which further decreases NO synthesis and increases oxidative stress in hypertension. The mechanism of increased superoxide production in aged vessels is not well understood and this may be complicated by accompanying pathological conditions. Similar to the endothelial dysfunction found in hypertension, a brief increase in intravascular pressure in isolated arterioles of young and normotensive rats reduced NO mediated flow-induced dilation through an increased superoxide production. High pressure or stretch enhances superoxide production through activation of NAD(P)H oxidase. Aging and high pressure act synergistically to cause a greater increase in superoxide production particularly in the endothelium. This diminishes NO bioavailability and causes endothelial dysfunction. Accordingly, the use of antioxidants to reduce oxidative stress in various pathological conditions and aging would be a reasonable therapeutic strategy. However, clinical trials of vitamin E supplementation have shown not to have significant effects on cardiovascular outcomes. Chronic antioxidant supplementation impairs endothelial function by increasing eNOS uncoupling. Antioxidant supplementation alone is not sufficient enough to decrease vascular oxidative stress (Jacobson AK, Yan C, Gao Q, Rincon-Skinner T, Rivera A, Edwards J et al. Aging enhances pressure-induced arterial superoxide formation. Am J Physiol Heart Circ Physiol 2007, in press).

159. Role of eNOS in protection from increased oxidative stress. Increased oxidative stress is an important but not the only factor in the aging process. The sources for ROS include cellular mitochondria, activated NAD(P)H oxidases, and uncoupled eNOS. ROS derived from mitochondrial activity is scavenged by NO released from eNOS (Mohanakumar KP, Thomas B, Sharma SM, Muralikrishnan D, Chowdhury R, Chiueh CC. Nitric oxide: an antioxidant and neuroprotector. Ann N Y Acad Sci 2002; 962:389-401). Whole body periodic acceleration by releasing nitric oxide from eNOS through increased pulsatile shear stress

decreases oxidatrive stress. The bioactivity of superoxide reflects its rates of production by NAD(P)H-oxidases, xanthine oxidase, or other pathways and its inactivation by superoxide dismutases (SODs). In an inflammatory setting, mesangial cells shift the balance of reactive oxygen and nitrogen species to the NO side to circumvent deleterious effects of superoxide. They do so by two mechanisms, a down-regulation of superoxide formation by suppression of NA(P)DH oxidase 1 expression and by simultaneous upregulation of superoxide dismutating SODs. In the inflammatory state, this becomes a protective pathway even when only low amounts of NO are present within the inflamed tissue. Thus upregulation of eNOS with whole body periodic acceleration suppresses NA(P)DH oxidase 1 activity and upregulates SOS's, both actions reducing oxidative stress (Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000; 105:1631-1639; Pleskova M, Beck KF, Behrens MH, Huwiler A, Fichtlscherer B, Wingerter O et al. Nitric oxide down-regulates the expression of the catalytic NADPH oxidase subunit Nox1 in rat renal mesangial cells. FASEB J 2006; 20:139-141). The fine-tuning of the spatial and temporal superoxide and NO production determines the outcome of an inflammatory process and may offer therapeutic intervention strategies. Uncoupling of eNOS occurs when there is a deficiency of BH4, a co-factor in NO production. In this situation, eNOS produces preferentially superoxide rather than nitric oxide. This can be counteracted by increased shear stress to the endothelium that prodces synthesis of BH4 thereby restoring coupling of eNOS.

160. Inflammation. This is a complex host's normal defense reaction to insult and stress, both physiological and nonphysiological, like chemicals, drugs, oxidants, or a variety of microbial entities. Inflammation responses, whether acute or chronic, are activated by well-coordinated, sequential events controlled by humoral and cellular reactions. Reactive oxygen species (ROS), such as supeoxide, hydroxyl anions, peroxide and reactive nitrogen species (RNS) are heavily implicated in the inflammatory process. The deleterious effects of ROS/RNS are dependent on their concentration and the microenvironment in which ROS/RNS are released. Overproduced or uncontrolled ROS/RNS are a major causative factor in tissue inflammation.

161. The introduction of neutrophils, lymphocytes, and macrophages, which release ROS, RNS, and lytic enzymes further amplify the inflammatory reaction. Furthermore, various inflammatory genes are activated by the transcription factor, NF-κB, which is extremely sensitive to oxidants and many proinflammatory substances. NF-κB is increased in human aging partly the result of its activation by NA(P)DH oxidases which themselves are increased in aging. NF-κB activates gene expression of proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α, as well as the proinflammatory enzymes, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). COX-2 and NF-κB are upregulated during aging, and are suppressed by caloric restriction (CR). Several pieces of evidence show that CR can modulate NF-κB activation by its antioxidative action. The involvement of the inflammatory process in several diseases has long been known, but until a recent proposal, its implication in the aging process has been less appreciated. Other age-related inflammatory processes are the age-related alterations of vascular endothelial and smooth muscle cells, which lead to pathogenic expressions, as seen in atherosclerosis and endothelial dysfunctions in diabetes. The evidence accumulated to date on the beneficial, antiaging action of CR is consistent with its anti-inflammatory property. The activation of age related NF-κB and the gene expression of several proinflammatory proteins are

all attenuated by caloric restriction (CR). The regulation of inflammatory response by CR at molecular levels is further demonstrated by suppression of age-related increases in proinflammatory COX-2 gene expression and PG synthesis. A similar attenuation by CR was shown on other NF-κB-dependent genes, IL-1ß, IL-6, TNF-α, COX-2, and iNOS. Thus, the inflammatory process is intricately involved in the aging process (Chung HY, Kim HJ, Kim JW, Yu BP. The inflammation hypothesis of aging: molecular modulation by calorie restriction. Ann N Y Acad Sci 2001; 928:327-335; Clark RA, Valente AJ. Nuclear factor kappa B activation by NADPH oxidases. Mech Ageing Dev 2004; 125:799-810; Donato AJ, Eskurza I, Silver AE, Levy AS, Pierce GL, Gates PE et al. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. Circ Res 2007; 100:1659-1666).

162. Overnutrition. Caloric restriction (CR) remains the only known robust means of increasing life span and delaying age-related physiological changes, and the onset of a wide range of age-related diseases in rodents. The enhanced metabolic efficiency that CR produces and the ability of CR to increase stress resistance over the lifespan have been hypothesized to be at the core of CR's anti-aging effects. Accumulating evidence clearly indicates that CR protects homeostatic integrity by prioritizing energy allocation to increase the resistance capacity against both intrinsic and extrinsic insults. Among its diverse effects, the anti-inflammatory action of CR at molecular levels through its modulation of oxidative stress is particularly noteworthy. As noted above, CR provides protection from the aging process by reducing oxidative stress and inflammation (Chung HY, Kim HJ, Kim KW, Choi JS, Yu BP. Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. Microsc Res Tech 2002; 59:264-272).

163. Shortening of telomeres. Telomeres were established cytogenetically in the first half of the 20th century as functional chromosome 'caps', and it is now a quarter of a century since they were characterized in molecular terms as stretches of repetitive DNA with high G–C strand asymmetry. It has been hypothesized that telomeres should face an 'end-replication problem'; that is, they should shorten with each round of DNA replication owing to the loss of the most distal primer for lagging-strand synthesis. However, an enzyme (telomerase) was discovered carrying a small RNA with a sequence that can act as a template for the elongation of the G-rich DNA terminus. It was experimentally demonstrated that telomeres in normal human fibroblasts shortened progressively during culture *in vitro*. For the first time, a clear mechanism had been identified for a biological clock that could measure mitotic time and account for the phenomenon of cell replicative senescence. In cell replicative senescence, under standard culture conditions, cells cease division after a more-or-less-fixed number of cell divisions (commonly termed the 'Hayflick limit') that is a characteristic of the particular cell strain. The timing of senescence depends primarily on the replicative history of the cells (the number of elapsed cell divisions) and much less on the passage of chronological time.

164. Differences between cell strains in telomere-shortening rates under constant external stress are due to different capacities for antioxidative defense. The single most important factor for maintaining low intracellular peroxide concentrations and a low rate of telomere shortening in human fibroblasts appears to be the production and activity levels of superoxide dismutases. Preliminary evidence that telomere shortening *in vivo* is linked with

disease states in which oxidative stress plays a causative role comes from recent findings that short telomere length in lymphocytes is correlated with the incidence of vascular dementia, atherosclerosis or aplastic anaemia. These data are often seen as an indication of faster cell turnover in the stem-cell compartment in accordance with the idea of constant telomere shortening rates. However, oxidative stress is plays a strong causal role in all of these diseases, and systemic oxidative stress, whether caused by either an unusually low antioxidative capacity or an unusually high exposure to oxidative stressors, can explain this association. This suggests that telomere length might serve as a biomarker of cumulative exposure to stress and a prognostic indicator for risk of late-life diseases.

165. A proportion of the oxidative damage inflicted upon telomeres remains unrepaired and determines the amount of shortening in the next round of replication. This proportion is related to the total amount of damage in the bulk of the genome. Although most of that damage has been repaired, it is the residual, unrepaired fraction that determines the probability of mutation. Thus, telomere shortening counts not only cell divisions but also the cumulative probability of mutations occurring, and short telomeres trigger senescence in response to oxidative stress and mutational probability. Telomeres act as cellular 'sentinels' for genomic damage and remove 'dangerous' cells from further proliferation (von Zginicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002; 27:339-344).

166. **Basis for Utility of Claim 47.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration counteracts the major events associated with aging, e.g., increased oxidative stress and inflammation, thereby managing the aging process.

Claim 48

167. Claim 48 recites "wherein periodic acceleration is used to manage Sjogren's syndrome". Sjögren's syndrome (SS) is an autoimmune disease of exocrine glands, involving particularly salivary and lacrimal glands. Sjögren's syndrome may occur alone (primary SS), or in association with a variety of other connective tissue diseases (secondary SS). Sjögren's syndrome can be a systemic disease in which patients also have problems with vasculitis, arthritis, gastrointestinal tract, kidneys, lungs and muscles. Sjögren's syndrome is related to rheumatoid arthritis and systemic lupus erythematosus. There is a close relationship between cytokine expression in salivary gland tissue and the development and progression of this disease. Various cytokines, such as tumor necrosis factor–α (TNF-α), interleukin-1ß (IL-1ß), IL-2, and interferon-γ (IFN-γ), have been detected in the salivary glands of humans as well as experimental animals during the development of SS.

168. The expression of both matrix metalloproteinase 9 (MMP-9) and p65, one of the components of NF-κß, is up-regulated in SS acinar cells of the salivary glands located near infiltrated lymphocytes, where destruction of the acinar structure seems to occur, compared with both the expression in cells distant from the infiltrated lymphocytes and the expression in cells from normal salivary glands. MMP-9 is one of the causal molecules in the destruction of the acinar structure in the salivary glands of SS patients and that suppression of MMP-9 activity in acinar cells provides a therapeutic strategy for clinical improvement in SS salivary glands.

Inhibition of NF-κß activity present in SS patients diminishes MMP-9 expression thereby providing a means to treat such patients (Azuma M, Aota K, Tamatani T, Motegi K, Yamashita T, Ashida Y et al. Suppression of tumor necrosis factor alpha-induced matrix metalloproteinase 9 production in human salivary gland acinar cells by cepharanthine occurs via down-regulation of nuclear factor kappaB: a possible therapeutic agent for preventing the destruction of the acinar structure in the salivary glands of Sjogren's syndrome patients. Arthritis Rheum 2002; 46:1585-1594).

169. **Basis for Utility of Claim 48.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration inhibits nuclear factor kappa beta activity thereby protecting against destruction of the acinar structure in salivary glands from patients with Sjögren's syndrome.

Claim 49

170. Claim 49 recites "wherein periodic acceleration is used to manage the chronic phase of Lyme disease". Lyme disease is caused by the tick-borne spirochete Borrelia burgdogeri. During infection, B. burgdogeri spreads from the site of the tick bite in the skin to various secondary organs throughout the body, especially the skin, the joints, the heart, and the nervous system, and induces inflammatory lesions. It is a systemic infection whose manifestations are promoted by a variety of cytokines and chemokines. In vitro studies showed that B. burgdorferi and its outer surface lipoprotein OspA are able to induce the activation and nuclear translocation of NF-nB, primarily p50/p65 heterodimeric complexes. Concomitant with this finding, B. burgdorferi was found to induce the chemokines RANTES, MCP-1, IL-8, Gro-a, IFN-inducible protein 10, and various NF-nB regulated cytokines. The induction of these chemokines and other adhesion molecules by B. burgdorferi is blocked using NF-nB inhibitors, thus demonstrating the central role of NF-nB in the inflammatory responses during this infection (Bell S, Degitz K, Quirling M, Jilg N, Page S, Brand K. Involvement of NF-kappa B signalling in skin physiology and disease. Cell Signal 2003; 15:1-7; Ebnet K, Brown KD, Siebenlist UK, Simon MM, Shaw S. Borrelia burgdorferi activates nuclear factor-kappa B and is a potent inducer of chemokine and adhesion molecule gene expression in endothelial cells and fibroblasts. J Immunol 1997; 158:3285-3292).

171. **Basis for Utility of Claim 49.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration inhibits nuclear factor kappa beta activity thereby reducing the inflammatory response caused by Borrelia burgdogeri, the etiologic agent of Lyme disease.

Claim 50

172. Claim 50 is canceled.

Claim 51

173. Claim 51 recites "wherein periodic acceleration is used to improve mucocilary clearance and surfactant production, and to minimize lung damage associated with usual positive pressure mechanical ventilation".

174. Mucociliary clearance. Ciliostimulation induced by various transmitters is mediated by the release of nitric oxide (NO). Freshly obtained adenoid tissue explants were pre-treated with the nitric oxide synthase (NOS) inhibitor N^G-nitro L-arginine (L-NNA), to determine whether the ciliostimulators terbutaline, methacholine, substance P, and endothelin-1 require the release of NO to increase ciliary beat frequency (CBF) *in vitro*. The L-NNA pre-treatment affected the change in CBF induced by each of the ciliostimulators tested. Therefore, NO acts as an intermediate messenger in the ciliated epithelium in response to various transmitters and mediators. On the other hand, pre-treatment with the NOS inhibitor L-NNA did not affect ciliary response to the second messengers cAMP and cGMP, thus suggesting that NO dependent mechanisms do not constitute the sole pathway for the stimulation of ciliary function. Both eNOS and iNOS could produce NO in the healthy paranasal sinus mucosa and that eNOS probably plays a major role in production of NO. Therefore, NO is involved in the regulation of mucociliary motility in the human sinus mucosa (Kim JW, Min YG, Rhee CS, Lee CH, Koh YY, Rhyoo C et al. Regulation of mucociliary motility by nitric oxide and expression of nitric oxide synthase in the human sinus epithelial cells. Laryngoscope 2001; 111:246-250; Runer T, Lindberg S. Ciliostimulatory effects mediated by nitric oxide. Acta Otolaryngol 1999; 119:821-825).

175. The impairment of mucociliary clearance (MCC) contributes to the pathophysiology of airway diseases, such as asthma, chronic bronchitis and cystic fibrosis. Nitric oxide (NO) is known to stimulate MCC. Whole body periodic acceleration (WBPA) was employed in conscious sheep and its effects on tracheal mucus velocity (TMV), an index of MCC inestigated. The effect of (WBPA) on TMV was assessed serially for 2 h with and without pretreatment with the eNO synthase inhibitor, L-NAME (25 mg/ kg, Iv.). WBPA increased TMV to a maximum of 136 ± 9% (mean ± SE) of baseline at 1 h. This effect was completely blocked by L-NAME (89 ± 7%; $p<0.05$), suggesting that pGz has a stimulatory effect on MCC, which is in part mediated by eNO. The effects of WBPA on human neutrophil elastase (HNE) mediated MCC dysfunction was investigated by aerosolizing HNE and measuring TMV serially for 4 h post pGz with and without L-NAME. HNE reduced TMV to 55 ± 2% of baseline and this depression was reversed within 30 min of completing the 1-h WBPA treatment (112 ± 7%; $p<0.05$). This protective effect began to wane by 4h. L-NAME completely blocked WBPA-mediated protection. Therefore, WBPA has a protective effect against HNE induced MCC depression related to eNO release (Delacruz LI, Sabatier JR, Abraham WM. The effects of sinusoidal acceleration on normal and abnormal mucociliary clearance in sheep. Am.J.Respir.Crit.Care Med. 2[Abstract Issue], A131, 2005).

176. Surfactant. NO may either activate or inhibit the pulmonary surfactant system. Under conditions favoring generation of peroxynitrite, surfactant is degraded with formation of lipid peroxides, nitration of tyrosine residues of surfactant proteins, and with loss of the surface activity. Peroxynitrite additionally decreases the oxygen uptake and sodium transport

in alveolar type II cells. The presumed degradation products of peroxynitrite damage the surfactant complex. The presence of superoxide, transient metal, high concentrations of NO, oxygen, and the absence of thiol groups, urate, and ascorbate in the airways promote the destructive role of NO. NO may increase free oxygen radicals by releasing ferritin-bound iron that may become a transient metal, required for the generation of hydroxyl radicals. Thiols and nitrosothiols (consisting of mostly reduced or nitrosylated glutathione) in the epithelial lining fluid are likely to control NO homeostasis. By becoming nitrosylated as a result of a burst of peroxynitrite (or NO), the thiols neutralize the toxic effects of peroxynitrite. In addition, by slowly releasing NO, S-nitrosothiols are an additional source of NO.

177. NO also can improve surfactant function. When lung surfactant is cycled in vitro (ie, the air-liquid interface is cyclically compressed and expanded, mimicking breathing movements), the addition of NO to the gas phase improves the surface activity. NO delays the conversion of the large surfactant aggregates to small vesicles that no longer are surface-active. The mechanism and physiological significance of this effect remains to be studied. Being highly soluble in lipid and sparingly soluble in water, NO (unlike the water-soluble superoxide) concentrates within the surfactant structures and the membranes in general. By acting as a chainbreaking antioxidant, NO serves as an antioxidant. At the interfacial surfactant lining, NO protects against oxidation of lipids and hydrophobic proteins (Hallman M, Bry K. Nitric oxide and lung surfactant. Semin Perinatol 1996; 20:173-185).

178. Thus, whole body periodic acceleration by releasing NO from eNOS through increased pulsatile shear stress suppresses oxidative stress that is destructive to surfactant and promotes surfactant activity in the presence of breathing movements.

179. Mechanical ventilation. Overventilation (OV) stimulates the immune system similar to that elicited by bacterial LPS. The effects of OV and LPS on NF-κß activation, chemokine release, and cytokine release were nearly indistinguishable. These findings suggest that by causing volutrauma, OV may be as dangerous to patients as are bacterial infections. In addition, under certain conditions, cytokines may promote growth of bacteria. This means that inappropriate ventilation strategies may contribute to ventilator-associated pneumonia. The amounts of mediators released by OV were similar to those found after perfusion with high LPS concentrations, indicating that these quantities are biologically relevant. One of the initial steps after OV is activation of the transcription factor NF-κß. All of the genes of the cytokines and chemokines detected in response to OV contain the NF-κß consensus sequence, whereas the genes of those mediators that were not increased (IL-10, IFN-g, VEGF) lack this sequence. Thus, the mediator profile observed is consistent with NF-κß playing an important role in the signal transduction elicited by OV. Further support for an important role of NF-kB in OV-induced mediator release comes from the finding that pretreatment with steroids blocked both NF-κß activation and mediator release. OV appears to be capable of inducing a complete inflammatory response by stimulating the production of cytokines and chemokines that in turn attract and activate neutrophils. OV also causes release of TNF, IL-6, and prostacyclin in a dose-dependent manner. Those findings provided direct evidence that ventilation may trigger the release of immune mediators. Whole body periodic acceleration inhibits activation of NF-κß through the release of NO from eNOS activation by pulsatile shear stress.

180. **Basis for Utility of Claim 51.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration improves mucociliary clearance, protects against oxidative stress induced degradation of pulmonary surfactant, improves surfact activity during breathing, and inhibits nuclear factor kappa beta upregulation associated with mechanical overventilation activity thereby favorably affecting the preceding factors.

Claim 52

181. Claim 52 recites "wherein periodic acceleration is used to treat patients who have corticosteroid resistance and asthma or corticosteroid resistance and inflammatory bowel disease". Nitrosative stress and nitration of proteins in airway epithelium are responsible for corticosteroid resistance in severe (steroid-dependent and steroidresistant) asthma and their ineffectiveness in COPD, supporting the potential role of future therapeutic strategies aimed at regulating NO synthesis in asthma. It is likely that more selective and potent NO modulators (NOS inhibitors and NO donors) given on a regular basis may have clinical benefit. Besides the determination of the exact role of different NOS enzymes and other sources of NO in airways, NO modulators will be needed to assess their potential clinical use. A deficiency of NO derived from eNOS contributes to airway hyperresponsiveness to bradykinin in severe asthmatics treated with glucocorticoids and might also be induced by allergen exposition. Downregulation of eNOS activity increases endothelial adhesion and extravasation of leukocytes whereas overexpression of eNOS inhibits allergen-induced airway inflammation and hyperresponsiveness. Indeed, in asthmatic patients significant correlations are observed between allergen-induced airway hyperresponsiveness and both reduced expression of eNOS and increased expression of iNOS (Kharitonov SA. NOS: molecular mechanisms, clinical aspects, therapeutic and monitoring approaches. Curr Drug Targets Inflamm Allergy 2005; 4:141-149).

182. Since both oxidative stress and endothelial dysfunction play a part in corticosteroid resistance in asthmatic patients, whole body periodic acceleration to release NO from eNOS and to restore integrity of eNOS are indicated in corticosteroid-resistance.

183. Glucocorticoids are potent inhibitors of T cell activation and proinflammatory cytokines and are highly effective treatment for active inflammatory bowel disease (IBD). However, failure to respond, acutely or chronically, to glucocorticoid therapy is a common indication for surgery in IBD, with as many as 50% of patients with Crohn's disease (CD) and approximately 20% of patients with ulcerative colitis (UC) requiring surgery in their lifetime as a result of poor response to glucocorticoids. Approximately one-third of patients with CD are steroid dependent and one-fifth are steroid resistant while approximately one-quarter of patients with UC are steroid dependent and one-sixth are steroid resistant (Farrell RJ, Kelleher D. Glucocorticoid resistance in inflammatory bowel disease. J Endocrinol 2003; 178:339-346). The effects of glucocorticoids result largely from interference with proinflammatory transcription factors such as NF-κß and AP-1. Both transcription factors play a pivotal role in the immune response of chronic inflammatory bowel disease. Excessive and constitutive epithelial activation of NF-κß and AP-1 as well as of the upstream MAP-kinases JNK and p38 might be involved in steroid unresponsiveness of Crohn's disease. Although TNF-α is produced mainly by monocytes, epithelial cells are also able to secrete this cytokine. Based on the therapeutic

success of anti-TNF-α therapy, it is well established that TNF-α plays a key pathogenic role in Crohn's disease. TNF-α can directly impair epithelial barrier function. Patients with inflammatory bowel disease have an increased mucosal permeability that may lead to a chronically enhanced immune response to microbial antigens and abrogated self-tolerance. This might explain the high constitutive expression of transcription factors and MAP kinases in steroid-resistant Crohn's disease patients. As NF-κß and AP-1 are strongly activated by TNF-α, which itself is a target of both transcription factors, this scenario constitutes an autoamplification loop. Corticosteroid resistance is associated with high constitutive epithelial activation of NF-κß in concert with other proinflammatory mediators that all inhibit glucocorticoid receptor (GR) activity (Bantel H, Schmitz ML, Raible A, Gregor M, Schulze-Osthoff K. Critical role of NF-kappaB and stress-activated protein kinases in steroid unresponsiveness. FASEB J 2002; 16:1832-1834).

184. Since upregulation of NF-κß inhibits activity of glucocorticoid receptors in inflammatory bowel disease leads to corticosteroid resistance, whole body periodic acceleration to release NO from eNOS and to inhibit NF-κß are indicated in corticosteroid-resistant inflammatory bowel disease patients.

185. **Basis for Utility of Claim 52.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration suppresses factors responsible for corticosteroid resistance including oxidative stress, endothelial dysfunction and nuclear factor kappa beta activity.

Claim 53

186. Claim 53 recites "wherein periodic acceleration is used to treat chronic otitis media". Antibiotics have led to a dramatic decline in the incidence of life-threatening complications of otitis media (OM), such as meningitis or brain abscess. However, inner ear dysfunction secondary to chronic OM, including high-frequency sensorineural hearing loss or vertigo, is not uncommon. NTHI is a small, gram-negative bacterium, existing as a commensal organism in the human nasopharynx. Although NTHI rarely causes life-threatening infections, it is a clinically important pathogen since it is one of the underlying causes of OM in children and exacerbates chronic obstructive pulmonary disease in adults. Although NTHI is a gram-negative bacterium, it expresses molecules that activate not only Toll-like receptor 4 (TLR4) but also TLR2. The interactions of NTHI antigens with specific host molecules are likely to be involved in the transition of NTHI from a commensal to a pathogenic organism. NTHI induces activation NF-κß via TLR-2 pathway (Moon SK, Woo JI, Lee HY, Park R, Shimada J, Pan H et al. Toll-like receptor 2-dependent NF-kappaB activation is involved in nontypeable Haemophilus influenzae-induced monocyte chemotactic protein 1 up-regulation in the spiral ligament fibrocytes of the inner ear. Infect Immun 2007; 75:3361-3372).

187. Cholesteatoma is a chronic inflammatory process of the middle ear as a compication of chronic otitis media that leads to destruction of the soft tissues and bone structures of the ear. Cholesteatoma tissue is composed of proliferating, migrating and highly keratinizing epithelium known as matrix and of intensive inflammatory infiltrate (perimatrix). The latter contain several inflammatory cells, fibroblasts, APC, endothelia, etc. Cholesteatoma

is associated with chronic otitis media, manifested by foul-smelling leakage from the ear and rich microbial flora. Infected epidermal cells and inflammatory cell infiltrates are the source of production of high quantities of proinflammatory cytokines such as IL-1 and TNF-a. The latter may activate osteoclasts leading to bone destruction. Human toll-like receptors (TLR 1-10) are crucial in the induction and activation of innate immunity in the course of an infection. They are expressed mainly on the cells of the immune system, and also on some epithelia and endothelia. Their ligands so called pathogen associated molecular patterns are abundant on invading microbes. TLR-ligand binding results in cell signal transduction and subsequent production of various proinflammatory cytokines such as IL-1 and TNF-a. The interactions of keratinocyte's TLRs with microbial ligands, which are abundant in the microenvironment of cholesteatoma, induce intracellular signal transduction, leading to the activation of NF-κß with subsequent stimulation of genes particularly involved in the production of inflammatory cytokines and perhaps some anti-microbial agents (Szczepanski M, Szyfter W, Jenek R, Wrobel M, Lisewska IM, Zeromski J. Toll-like receptors 2, 3 and 4 (TLR-2, TLR-3 and TLR-4) are expressed in the microenvironment of human acquired cholesteatoma. Eur Arch Otorhinolaryngol 2006; 263:603-607). Inhibition of NF-κß activity with NO released from eNOS as with whole body periodic acceleration suppresses both IL-1 and TNF-a levels, thereby providing treatment for chronic otitis media (Garg A, Aggarwal BB. Nuclear transcription factor-kappaB as a target for cancer drug development. Leukemia 2002; 16:1053-1068).

188. **Basis for Utility of Claim 53.** The utility of this claim is based upon sound evidence that nitric oxide released from eNOS by whole body periodic acceleration inhibits nuclear factor kappa beta activity that is the key factor underlying chronic otitis media and its sequelae.

Claim 54

189. Claim 54 is canceled.

Claim 55

190. Claim 55 recites "wherein periodic acceleration is used to in conjunction with cell free hemoglobin transfusion in order to treat and/or prevent a nitric oxide deficit". The major driving force behind hemoglobin-based blood substitute research is the need for an unlimited and safe supply of an O2 delivery fluid for resuscitation after traumatic blood loss (hemorrhagic shock) and transfusions after elective surgery. In principle, Hb-based oxygen carrier products could also be used to treat ischemia due to strokes and myocardial infarctions because small, extracellular Hb molecules (diameter c0.006 to 0.012 Am) can flow around partial arterial blockages and oxygenate the surrounding tissue. However, the vasoconstrictive activities of most current products prevent their consideration for the latter application. All first-generation Hb-based blood substitute products cause elevation of arterial blood pressure, some gastrointestinal discomfort and loss of motility, and reversible myocardial lesions.

191. The most widely accepted interpretation of the blood pressure effect is rapid scavenging of NO by extracellular hemoglobin, which prevents smooth muscle relaxation. HbO2 in red cells has been shown to oxidize any NO that enters the bloodstream as a result of

inhalation. However, the rate of NO consumption by whole blood is limited by diffusion up to and into the red cells and is too slow to interfere significantly with vasoregulation during normal flow rates. In contrast, extracellular HbO2 is much more vasoconstrictive for two reasons. First, in isotropic solutions the intrinsically high rate of reaction of HbO2 with NO is not limited by diffusion through unstirred layers adjacent to the erythrocyte membrane or through cell-free layers generated at the vessel walls during rapid blood flow. The small hemoglobin molecules can stream next to arterial walls and serve as an NO "sink". Rapid consumption of NO at the endothelial surface will skew any NO diffusion gradient produced by endothelial NO synthase toward the lumen of the vessel and away from the ablumenal guanylyl cyclase receptors located in smooth muscle. Second, and probably more important, hemoglobin tetramers can extravasate rapidly into the blood vessel wall and consume nitric oxide in the interstitial space between the endothelium and the smooth muscle, directly disrupting the NO signaling process. In either case, this process leads to nitric oxide deficiency at the level of eNOS (Olson JS, Foley EW, Rogge C, Tsai AL, Doyle MP, Lemon DD. No scavenging and the hypertensive effect of hemoglobin-based blood substitutes. Free Radic Biol Med 2004; 36:685-697).

192. **Basis for Utility of Claim 55.** The utility of this claim is based upon the fact that nitric oxide released from eNOS by whole body periodic acceleration corrects nitric oxide deficiency induced by cell free hemoglobin transfusion.

Claim 56

193. Claim 56 is canceled.

Conclusion

194. The above statements provide adequate evidence of the utility of each of claims 18-32, 35-36, 38-49, 51-53, and 55.

195. Claims 59-73, 76-77, 79-90, 92-94, and 96 correspond to claims 18-32, 35-36, 38-49, 51-53, and 55, resepectively. Accordingly, the above statements also provide evidence of the utility for these corresponding claims.

196. I hereby declare that all statements made herein of my own knowledge are true, all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful statements may jeopardize the validity of this patent application or any patent resulting therefrom.

August 3, 2007
Date

Marvin Sackner, MD
Dr. Marvin Sackner

APPENDIX A

NF-kB DISEASES

On this page are listed several diseases in which activation of NF-kB has been implicated. For general reviews on the role of NF-kB in disease, see Aradhya & Nelson (2001), Kumar et al (2004), Yamamoto & Gaynor (2002) or Baldwin (2001). For specific diseases see the listed references, which can be found either on this site (under References) or at PubMed (through the linked references).

Table 1: General diseases

	Review
Ageing	Chung et al, 2002
Headaches	Reuter et al, 2003
Pain	Tegeder et al, 2004
Cardiac Hypertrophy	Purcell & Molkentin, 2003
Muscular Dystrophy (type 2A)	Baghdiguian et al, 1999
Catabolic disorders	Holmes-McNary, 2002
Diabetes, Type 1	Ho & Bray, 1999
Diabetes, Type 2	Yuan et al, 2001; Lehrke et al, 2004; Chen, 2005
Hypercholesterolemia	Wilson et al, 2000
Atherosclerosis	Ross et al, 2001; Li & Gao, 2005
Heart Disease	Valen et al, 2001
Chronic Heart Failure	Frantz et al, 2003
Ischemia/reperfusion	Toledo-Pereyra & Lopez-Neblina, 2002; Nichols, 2004
Stroke	Herrmann et al, 2005
Angina Pectoris	Ritchie, 1998
Pulmonary Disease	Christman et al, 2000
Cystic Fibrosis	Pollard et al, 2005
Acid-induced Lung Inury	Madjdpour et al, 2003
Chronic Obstructive Pulmonary Disease (COPD)	Barnes, 2002
Hyaline Membrane Disease	Cheah et al, 2005
Renal Disease	Guijarro & Egido, 2001
Glomerular Disease	Zheng et al, 2005

Leptospiriosis renal disease	Yang et al, 2001
Gut Diseases	Neurath et al, 1998
Skin Diseaes	Bell et al, 2003
Anhidrotic Ecodermal Dysplasia-ID	Puel et al, 2005
Behcet's Disease	Todaro et al, 2005
Incontinentia pigmenti	Courtois & Israel, 2000
Asthma	Pahl & Szelenyi, 2002
Arthritis	Roshak et al, 2002
Crohn's Disease	Pena & Penate, 2002
Colitis (rat)	Chen et al, 2005
Ocular Allergy	Bielory et al, 2002
Glaucoma	Zhou et al, 2005
Appendicitis	Pennington et al, 2000
Pancreatitis	Weber & Adler, 2001
Periodonitis	Nichols et al, 2001; Ambili et al, 2005
Inflammatory Bowel Disease	Dijkstra et al, 2002
Sepsis	Wratten et al, 2001; Abraham, 2003
Silica-induced	Chen & Shi, 2002
Sleep apnoea	Lavie, 2003
AIDS (HIV-1)	Hiscott et al., 2001
Autoimmunity	Hayashi & Faustman, 2000; Bacher & Schmitz, 2004
Lupus	Kammer & Tsokos, 2002
Psychosocial stress diseases	Bierhaus et al, 2004
Neuropathological Diseases	Cechetto, 2001; Mattson & Camandola, 2001
Familial amyloidotic polyneuropathy, inflamm neuropathy	Mazzeo et al, 2004
Traumatic brain injury	Hang et al, 2005
Parkinson Disease	Soos et al, 2004
Alzheimers Disease	Mattson & Camandola, 2001
Huntington's Disease	Khoshnan et al, 2004
Retinal Disease	Kitaoka et al, 2004
Sudden hearing loss	Merchant et al, 2005

Cancer	Gilmore et al, 2002; Karin et al, 2002 (see Table 2, below)

Table 2:

Cancer type	*Reference*
A: Primary tumors and tumor cell lines	
	Nakshatri et al, 1997; Sovak et al, 1997
Cervix	Nair et al, 2003; Kumar et al, 2005
Ovary	Dejardin et al, 1999; Huang et al, 2000
Vulva	Seppanen & Vihko, 2000
Prostate	Huang et al, 2001; Palayoor et al, 1999; Fradet et al, 2004
Kidney	Oya et al, 2001, 2003
Bladder	Horiguchi et al, 2003
Lung	Tichelaar et al, 2004
Liver	Tai et al, 2000, Arsura & Cavin, 2005
Pancreas	Wang et al, 1999; Sclabas et al, 2003; Xiong, 2004
Esophygeal/gastric	Sutter et al, 2004; Lee et al, 2005; Abdel-Latif et al, 2005
Laryngeal	Zhu et al, 2004; Pan et al, 2005
Stomach	Sasaki et al, 2001
Colon	Lind et al, 2001
Thyroid	Visconti et al, 1997; Pacifico et al, 2004
Melanoma	Yang & Richmond, 2001; Torabian & Kashani-Sabet, 2005; Amiri & Richmond, 2005
Squamous cell carcinoma	Loercher et al, 2004
Head and neck	Ondrey et al, 1999; Chang & Van Waes, 2005
Endometrial	Pallares et al, 2004
Cylindromatosis	Kovalenko et al, 2003; Brummelkamp et al, 2003; Trompouki et al, 2003
Hilar Cholangiocarcinoma	Chen et al, 2005
Oral carcinoma	Nakayama et al, 2001
Astrocytoma/glioblastoma	Hayahsi et al, 2001; Garkavtsev et al, 2004

Neuroblastoma	Bian et al, 2002
Hodgkin's lymphoma	Bargou et al, 1996, 1997; Staudt, 2000
Acute lymphoblastic leukemia	Kordes et al, 2000; Munzert et al, 2004
Acute myelogenous leukemia	Guzman et al, 2001
Acute T-cell leukemia (HTLV-1)	Arima & Tei, 2001
Chronic lymphocytic leukemia	Furman et al, 2000
Burkitts Lymphoma (EBV)	Knecht et al, 2001
Mantle cell lymphoma	Martinez et al, 2003
Multiple myeloma	Berenson et al, 2001
Diffuse large B-cell lymphoma	Davis et al, 2001; Shaffer et al, 2002
B: In vitro transformation of cells	
AP12/MALT1	Stoffel et al, 2004; Ho et al, 2005
BCR-ABL	Reuther et al, 1999
DBL/DBS	Whitehead et al, 1999
Pim-2	Fox et al, 2003; Hammerman et al, 2004
PDGF-beta chain	Shimamura et al, 2002
RAF	Baumann et al, 2000
RAS	Finco et al, 1997
RET/PTC3	Russell et al, 2003
TEL-JAK2	Santos et al, 2001
TEL-PDGFR	Besancon et al, 1998
Vav	Palmby et al, 2004

Reference List

1. Abdel-Latif MM, O'Riordan JM, Ravi N, Kelleher D & Reynolds JV (2005) Activated nuclear factor-k B and cytokine profiles in the esophagus parallel tumor regression following neoadjuvant chemoradiotherapy. Diseases of Esophagus 18: 246-252

2. Abraham E (2003) Nuclear factor-kB and its role in sepsis-associated organ failure. Journal of Infectious Diseases 187: S364-S369

3. Ambili R, Santhi WS, Janam P, Nandakumar K and Pillai MR (2005) Expression of activated transcription factor nuclear factor-kB in periodontally diseased tissues. Journal of Periodontology 76: 1148-1153

5. Amiri KI and Richmond A (2005) Role of nuclear factor-kB in melanoma. Cancer Metastasis Reviews 24:

301-313

5. Aradhya S and Nelson DL (2001) NF-kB signaling and human disease. Current Opinions in Genetics and Development 11: 300-306

6. Arima N and Tei C (2001) HTLV-I Tax related dysfunction of cell cycle regulators and oncogenesis of adult T cell leukemia. Leukemia and Lymphoma 40: 267-278

7 Arsura M and Cavin LG (2005) Nuclear factor-kB and liver carcinogenesis. Cancer Letters 229: 157-169

8. Bacher S and Schmitz ML (2004) The NF-kB pathway as a potential target for autoimmune disease therapy. Current Pharmeutical Design 10: 2827-2837

9. Baghdiguian S, Martin M, Richard I, Pons F, Astier C, Bourg N, Hay RT, Chemaly R, Halaby G, Loiselet J, Anderson LV, Lopez de Munain A, Fardeau M, Mangeat P, Beckmann JS and Lefranc G (1999) Calpain 3 deficiency is associated with myonuclear apoptosis and profound perturbation of the IkB alpha/NF-kB pathway in limb-girdle muscular dystrophy type 2A . Nature Medicine 5: 503-511 [published erratum appears in Nat Med 1999 Jul;5(7):849]

10. Baldwin AS Jr (2001) Series introduction: the transcription factor NF-kB and human disease. Journal of Clinical Investigation 107: 3-6

11. Bargou RC, Leng C, Krappmann D, Emmerich F, Mapara MY, Bommert K, Royer H-D, Scheidereit C and Dörken (1996) High-level nuclear NF-kB and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. Blood 87: 4340-4347

12. Bargou RC, Emmerich F, Krappmann D, Bommert K, Mapara MY, Arnold W, Royer HD, Grinstein E, Greiner A, Scheidereit C and Dörkin B (1997) Constitutive NF-kB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. Journal of Clinical Investigation 100: 2961-2969

13. Barnes PJ (2002) New treatments for COPD. Nature Reviews Drug Discovery 1: 437-446

14. Baumann B, Weber CK, Troppmair J, Whiteside S, Israel A, Rapp UR and Wirth T (2000) Raf induces NF-kB by membrane shuttle kinase MEKK1, a signaling pathway critical for transformation. Proceedings of the National Academy of Sciences USA 97: 4615-4620

15. Bell S, Degitz K, Quirling M, Jilg N, Page S and Brand K (2003) Involvement of NF-kB signalling in skin physiology and disease. Cell Signal 15: 1-7

16. Berenson JR, Ma HM and Vescio R (2001) The role of nuclear factor-kB in the biology and treatment of multiple myeloma. Seminars in Oncology 28: 626-633

17. Besancon F, Atfi A, Gespach C, Cayre YE and Bourgeade MF (1998) Evidence for a role of NF-kB in the survival of hematopoietic cells mediated by interleukin-3 and the oncogenic TEL/platelet-derived growth factor beta fusion protein. Proceedings of the National Academy of Sciences USA 95: 8081-8086

18. Bian X, Opipari Jr AW, Ratanaproeksa AB, Boltano AE, Lucas PC and Castle VP (2002) Constitutively active NF-kB is required for the survival of S-type neuroblastoma. Journal of Biological Chemistry 277: 42144-42150

19. Bielory L, Kempuraj D and Theoharides T (2002) Topical immunopharmacology of ocular allergies. Current Opinion in Allergy and Clinical Immunology 2: 435-445

20. Bierhaus A, Humpert PM and Nawroth PP (2004) NF-kB as a molecular link between psychosocial stress and organ dysfunction. Pediatic Nephrology 19: 1189-1191

21. Brummelkamp TR, Nijman SMB, Cirac AMG and Bernards R (2003) Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kB. Nature 424: 797-801

22. Cechetto DF (2001) Role of nuclear factor kappaB in neuropathological mechanisms. Progress in Brain

Research 132: 391-404

23. Chang AA and Van Waes C (2005) Nuclear factor-kB as a common target and activator of oncogenes in head and neck squamous cell carcinoma. Advances in Otorhinolaryngology 62: 92-102

24. Cheah FC, Winterbourn CC, Darlow BA, Mocatta TJ and Vissers MC (2005) Nuclear factor kB activation in pulmonary leukocytes from infants with hyaline membrane disease: associations with chorioamnionitis and ureaplasma urealyticum colonization. Pediatric Research 57: 616-623

25. Chen F (2005) Is NF-kB a culprit in type 2 diabetes? Biochemical and Biophysical Research Communications 332: 1-3

26. Chen F and Shi X (2002) NF-kB, a pivotal transcription factor in silica-induced diseases. Molecular and Cellular Biochemistry 234-235: 169-176

27. Chen K, Long YM, Wang H, Lan L and Liu ZH (2005) Activation of nuclear factor-kappa B and effects of pyrrolidine dithiocarbamate on TNBS-induced rat colitis. World Journal of Gastroenterology 11: 1508-1514

28. Chen RF, Li ZH, Kong XH and Chen JS (2005) Effect of mutated IkBa transfection on multidrug resistance in hilar cholangiocarcinoma cell lines. World Journal of Gastroenterology 11: 726-728

29. Christman JW, Sadikot RT and Blackwell TS (2000) The role of nuclear factor-kB in pulmonary diseases. Chest 117: 1482-1487

30. Chung HY, Kim HJ, Kim KW, Choi JS and Yu BP (2002) Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. Microscopy Research and Technique 59: 264-272

31. Courtois G and Israel A (2000) NF-kB defects in humans: the NEMO/incontinentia pigmenti connection. Sci STKE Nov 14 (58): PE1

32. Davis RE, Brown KD, Siebenlist U and Staudt LM (2001) Constitutive nuclear factor kB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. Journal of Experimental Medicine 194: 1861-1874

33. Dejardin E, Deregowski V, Chapelier M, Jacobs N, Gielen J, Merville M-P and Bours (1999) Regulation of NF-kB activity by IkB-related proteins in adenocarcinoma cells. Oncogene 18: 2567-2577

34, Dijkstra G, Moshage H and Jansen PL (2002) Blockade of NF-kB activation and donation of nitric oxide: new treatment options in inflammatory bowel disease? Scandinavian Journal of Gastroenterology Suppl 236: 37-41

35. Finco TS, Westwick JK, Norris JL, Beg AA, Der CJ and Baldwin Jr AS (1997) Oncogenic Ha-Ras-induced signaling activates NF-kB transcriptional activity, which is required for cellular transformation. Journal of Biological Chemistry 272: 24113-24116

36. Fox CJ, Hammerman PS, Cinalli RM, Master SR, Chodosh LA and Thompson CB (2003) The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptotic inhibitor. Genes & Development 17: 1841-1854

37. Fradet V, Lessard L, Begin LR, Karakiewicz P, Masson AM and Saad F (2004) Nuclear factor-kB nuclear localization is predictive of biochemical recurrence in patients with positive margin prostate cancer. Clinical Cancer Research 10: 8460-8464

38. Frantz S, Fraccarollo D, Wagner H, Behr TM, Jung P, Angermann CE, Ertl G, Bauersachs J (2003) Sustained activation of nuclear factor kappaB and activator protein 1 in chronic heart failure. Cardiovascular Research 57: 749-756

39. Furman RR, Asgary Z, Mascarenhas JO, Liou HC and Schattner EJ (2000) Modulation of NF-kB activity and apoptosis in chronic lymphocytic leukemia B cells. Journal of Immunology 164: 2200-2206

40. Garkavtsev I, Kozin SV, Chernova O, Xu L, Winkler F, Brown E, Barnett GH and Jain RK (2004) The candidate tumour suppressor protein ING4 regulates brain tumour growth and angiogenesis. Nature 428: 328-332

41. Gilmore TD, Gapuzan M-E, Kalaitzidis D and Starczynowski D (2002) Rel/NF-kB/IkB signal transduction in the generation and treatment of human cancer. Cancer Letters 181: 1-9

42. Guijarro C and Egido J (2001) Transcription factor-kappaB (NF-kB) and renal disease. Kidney International 59: 415-424

43. Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, Luger SM and Jordan CT (2001) Nuclear factor-kB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 98: 2301-2307

44. Hammerman PS, Fox CJ, Cinalli RM, Xu A, Wagner JD, Lindsten T and Thompson CB (2004) Lymphocyte transformation by Pim-2 is dependent on nuclear factor-kB activation. Cancer Research 64: 8341-8348

45. Hang CH, Shi JX, Li JS, Li WQ and Yin HX (2005) Up-regulation of intestinal nuclear factor kB and intercellular adhesion molecule-1 following traumatic brain injury in rats. World Journal of Gastroenterology 11: 1149-1154

46. Hayashi T and Faustman D (2002) Defective function of the proteasome in autoimmunity: involvement of impaired NF-kB activation. Diabetes Technol Ther 2: 415-428

47. Hayashi S, Yamamoto M, Ueno Y, Ikeda K, Ohshima K, Soma G and Fukushima T (2001) Expression of nuclear factor-kB, tumor necrosis factor receptor type 1, and c-Myc in human astrocytomas. Neurologia Medico-Chirufica 41: 187-195

48. Herrmann O, Baumann B, de Lorenzi R, Muhammad S, Zhang W, Kleesiek J, Malfertheiner M, Kohrmann M, Potrovita I, Maegele I, Beyer C, Bruke JR, Hasan MT, Bujard H, Wirth T, Pasparakis M and Schwaninger M (2005) IKK mediates ischemia-induced neuronal death. Nature Medicine: in press

49. Hiscott J, Kwon H and Genin P (2001) Hostile takeovers: viral appropriation of the NF-kB pathway. Journal of Clinical Investigation 107: 143-151

50. Ho E and Bray TM (1999) Antioxidants, NFkB activation, and diabetogenesis. Proceedings of the Society of Experimental Biology and Medicine 222: 205-213

51. Ho L, Davis RE, Conne B, Chappuis R, Berczy M, Mhawech P, Staudt LM and Schwaller J (2005) MALT1 and the API2-MALT1 fusion act between CD40 and IKK and confer NF-kB dependent proliferative advantage and resistance against FAS-induced cell death in B cells. Blood 105: 2891-2899

52. Holmes-McNary M (2002) Nuclear factor kappaB signaling in catabolic disorders. Current Opinion in Clinical Nutrition and Metabolic Care 5: 255-263

53. Horiguchi Y, Kuroda K, Nakashima J, Murai M and Umezawa K (2003) Antitumor effect of a novel nuclear factor-kappaB activation inhibitor in bladder cancer cells. Expert Reviews in Anticancer Therapy 3: 793-798

54. Huang S, Pettaway CA, Uehara H, Bucana CD and Fidler IJ (2001) Blockade of NF-kB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. Oncogene 20: 4188-4197

55. Huang S, Robinson JB, DeGuzman A, Bucanna CD and Fidler IJ (2000) Blockade of nuclear factor-kB signaling inhibits angiogenesis and tumorigenicity of ovarian cancer cells by suppressing expression of vascular endothelial growth factor and Interleukin 8. Cancer Research 60: 5334-5339

56. Karin M, Cao Y, Greten FR and Li ZW (2002) NF-kB in cancer: from innocent bystander to major culprit.

Nature Reviews Cancer 2: 301-310

57. Kammer GM and Tsokos GC (2002) Abnormal T lymphocyte signal transduction in systemic lupus erythematosus. Current Directions in Autoimmunity 5: 131-150

58. Khoshnan A, Ko J, Watkin EE, Paige LA, Reinhart PH and Patterson PH (2004) Activation of the IkB kinase complex and nuclear factor-kB contributes to mutant Huntingtin neurotoxicity. Journal of Neuroscience 24: 7999-8008

59. Kitaoka Y, Kumai T, Kitaoka Y, Lam TT, Munemasa Y, Isenoumi K, Motoki M, Kuribayashi K, Kogo J, Kobayashi S and Ueno S (2004) Nuclear factor-kB p65 in NMDA-induced retinal neurotoxicity. Brain Research Molecular Brain Research 131: 8-16

60. Knecht H, Berger C, Rothenberger S, Odermatt BF and Brousset P (2001) The role of Epstein-Barr virus in neoplastic transformation. Oncology 60: 289-302

61. Kordes U, Krappmann D, Heissmeyer V, Ludwig WD and Scheidereit C (2000) Transcription factor NF-kB is constitutively active in acute lymphoblastic leukemia cells. Leukemia 14: 399-402

62. Kovalenko A, Chabie-Bessia C, Cantarella G, Israel A, Wallach D and Courtois G (2003) The tumour suppressor CYLD negatively regulates NF-kB signalling by deubiquitination. Nature 424: 801-805

63. Kumar A, Takada Y, Boriek AM and Aggarwal BB (2004) Nuclear factor-kB: its role in health and disease. Journal of Molecular Medicine 82: 434-448

64. Kumar B, Husain SA and Chandra B (2005) Constitutive activation of nuclear factor-kB: Preferential homodimerization of p50 subunits in cervical carcinoma. Frontiers in Bioscience 10: 1510-1519

65. Lavie L (2003) Obstructive sleep apnoea syndrome - an oxidative stress disorder. Sleep Medicine Review 7: 35-51

66. Lee BL, Lee HS, Jung J, Cho SJ, Chung HY, Kim WH, Jin YW, Kim CS and Nam SY (2005) Nuclear factor-kB activation correlates with better prognosis and Akt activation in human gastric cancer. Clinical Cancer Research 11 2518-2525

67. Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ and Lazar MA (2004) An inflammatory cascade leading to hyperresistinemia in humans. Plos Med 1: e45

68. Li JJ and Gao RL (2005) Should atherosclerosis be considered a cancer of the vascular wall? Medical Hypotheses 64: 694-698

69. Lind DS, Hochwald SN, Malaty J, Rekkas S, Hebig P, Mishra G, Moldawer LL, Copeland EM 3rd and Mackay S (2001) Nuclear factor-kB is upregulated in colorectal cancer. Surgery 130: 363-369

70. Loercher A, Lee TL, Ricker JL, Howard A, Geoghegen J, Chen Z, Sunwoo JB, Sitcheran R, Chuang EY, Mitchell JB, Baldwin AS Jr and Van Waes C (2004) Nuclear factor-kB is an important modulator of the altered gene expression profile and malignant phenotype in squamous cell carcinoma. Cancer Research 64: 6511-65239

71. Madjdpour L, Kneller S, Booy C, Pasch T, Schimmer RC and Beck-Schimmer B (2003) Acid-induced lung injury: role of Nuclear Factor-kB. Anesthesiology 99: 1323-1332

72. Martinez N, Camacho FI, Algara P, Rodriguez A, Dopazo A, Ruiz-Ballesteros E, Martin P, Martinez-Climent JA, Garcia-Conde J, Menarguez J, Solano F, Mollejo M and Piris MA (2003) The molecular signature of mantle cell lymphoma reveals multiple signals favoring cell survival. Cancer Research 63: 8226-8232

73. Mattson MP and Camandola S (2001) NF-kB in neuronal plasticity and neurodegenerative disorders. Journal of Clinical Investigation 107: 247-254

74. Mazzeo A, Aguennouz M, Messina C and Vita G (2004) Immunolocalization and activation of transcription factor nuclear factor kB in dysimmune neuropathies and familial amyloidotic polyneuropathy. Archives of Neurology 61: 1097-1102

75. Merchant SN, Adams JC and Nadol JB Jr (2005) Pathology and pathophysiology of idiopathic sudden sensorineural hearing loss. Otology and Neurotology 26: 151-160

76. Munzert G, Kirchner D, Ottmann O, Bergmann L and Schmid RM (2004) Constitutive NF-kB/Rel activation in philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL). Leukemia and Lymphoma 45: 1181-1184

77. Nair A, Venkatraman M, Maliekal TT, Nair B and Karunagaran D (2003) NF-kB is constitutively activated in high- grade squamous intraepithelial lesions and squamous cell carcinomas of the human uterine cervix. Oncogene 22: 50-58

78. Nakayama H, Ikebe T, Beppu M and Shirasuma K (2001) High expression levels of nuclear factor kB, IkB kinase b and Akt kinase in squamous cell carcinoma of the oral cavity. Cancer 92: 3037-3044

79. Nakshatri H, Bhat-Nakshatri P, Martin DA, Goulet Jr RJ and Sledge Jr GW (1997) Constitutive activation of NF-kB during progression of breast cancer to hormone-independent growth. Molecular and Cellular Biology 17: 3629-3639

80. Neurath MF, Becker C and Barbulescu K (1998) Role of NF-kB in immune and inflammatory responses in the gut. Gut 43: 856-860

81. Nichols TC, Fischer TH, Deliargyris EN and Baldwin Jr AS (2001) Role of nuclear factor-kappaB (NF-kB) in inflammation, periodontitis, and atherogenesis. Annals of Periodontology 6: 20-29

82. Nichols TC (2004) NF-kB and reperfusion injury. Drug News and Perspectives 17: 99-104

83. Ondrey FG, Dong G, Sunwoo J, Chen Z, Wolf JS, Crowl-Bancroft CV, Mukaida N and Van Waes C (1999) Constitutive activation of transcription factors NF-kB, AP-1, and NF-IL6 in human head and neck squamous cell carcinoma cell lines that express pro-inflammatory and pro-angiogenic cytokines. Molecular Carcinogenesis 26: 119-129

84. Oya M, Ohtsubo M, Takayanagi A, Tachibana M, Shimizu N and Murai M (2001) Constitutive activation of NF-kB prevents TRAIL-induced apoptosis in renal cancer cells. Oncogene 20: 3888-3896

85. Oya M, Takayanagi A, Horiguchi A, Mizuno R, Ohtsubo M, Marumo K, Shimizu N and Murai M (2003) Increased nuclear factor kappaB activation is related to tumor development of renal cell carcinoma. Carcinogenesis 24: 377-384

86. Pacifico F, Mauro C, Barone C, Crescenzi E, Mellone S, Monaco M, Chiappetta G, Terrazzano G, Liguoro D, Vito P, Consiglio E, Formisano S and Leonardi A (2004) Oncogenic and anti-apoptotic activity of NF-kB in human thyroid carcinomas. Journal of Biological Chemistry 279: 54610-54619

87. Pahl A and Szelenyi I (2002) Asthma therapy in the new millennium. Inflammation Research 51: 273-282

88. Palayoor ST, Yourmell MY, Calderwood SK, Coleman CN and Price BD (1999) Constitutive activation of IkB kinase b and NF-kB in prostate cancer cells is inhibited by ibuprofen. Oncogene 18: 7389-7394

89. Pallares J, Martinez-Guitarte JL, Dolcet X, Llobet D, Rue M, Palacios J, Prat J and Matias-Guiu X (2004) Abnormalities in the NF-kB family and related proteins in endometrial carcinoma. Journal of Pathology 204: 595-577

90. Palmby TR, Abe K, Karnoub AE and Der CJ (2005) Vav transformation requires activation of multiple GTPases and regulation of gene expression. Molecular Cancer Research 2: 702-711

91. Pan S, Tao Z, Wu L, Xiao B and Chen S (2005) Nuclear factor kB/p65 and cyclooxygenase-2 expression

and clinic significance in human laryngeal squamous cell carcinoma [Article in Chinese]. Lin Chuang Er Bi Yan Hou Ke Za Zhi 19: 535-537

92. Pena AS and Penate M (2002) Genetic susceptibility and regulation of inflammation in Crohn's disease. Relationship with the innate immune system [Article in English, Spanish]. Rev Esp Enferm Dig 94: 351-360

93. Pennington C, Dunn J, Li C, Ha T and Browder W (2000) Nuclear factor kB activation in acute appendicitis: a molecular marker for extent of disease? American Surgery 66: 914-918

94. Pollard HB, Ji XD, Jozwik C and Jacobowitz DM (2005) High abundance protein profiling of cystic fibrosis lung epithelial cells. Proteomics 5: 2210-2216

95. Puel A, Yang K, Ku CL, von Bernuth H, Bustamante J, Santos OF, Lawrence T, Chang HH, Al-Mousa H, Picard C and Casonova JL (2005) Heritable defects of the human TLR signalling pathways. Journal of Endotoxin Research 11: 220-224

96. Purcell NH and Molkentin JD (2003) Is nuclear factor kappaB an attractive therapeutic target for treating cardiac hypertrophy? Circulation 108: 638-640

97. Reuter U, Chiarugi A, Bolay H and Moskowitz MA (2003) Nuclear factor-kB as a molecular target for migraine therapy. Headache 43: 426-427

98. Reuther JY, Reuther GW, Cortez D, Pendergast AM and Baldwin Jr AS (1998) A requirement for NF-kB activation in Bcr-Abl mediated transformation. Genes & Development 12: 968-981

99. Ritchie ME (1998) Nuclear factor kappaB is markedly and selectively activated in humans with unstable angina pectoris. Circulation 98: 1707-1713

100. Roshak AK, Callahan JF and Blake SM (2002) Small-molecule inhibitors of NF-kB for the treatment of inflammatory joint disease. Current Opinion in Pharmacology 2: 316-321

101. Ross JS, Stagliano NE, Donovan MJ, Breitbart RE and Ginsburg GS (2001) Atherosclerosis: a cancer of the blood vessels? American Journal of Clinical Pathology 116: S97-S107

102. Russell JP, Shinohara S, Melillo RM, Castellone MD, Santoro M and Rothstein JL (2003) Tyrosine kinase oncoprotein, RET/PTC3, induces the secretion of myeloid growth and chemotactic factors. Oncogene 22: 4569-4577

103. Santos SC, Monni R, Bouchaert I, Bernard O, Gisselbrecht S, Gouilleux F, Penard-Lacronique V (2001) Involvement of the NF-kB pathway in the transforming properties of the TEL-Jak2 leukemogenic fusion protein. FEBS Letters 497: 148-152

104. Sasaki N, Morisaki T, Hashizume K, Yao T, Tsuneyoshi M, Noshiro H, Nakamura K, Yamanaka T, Uchiyama A, Tanaka M and Katano M (2001) Nuclear factor-kB p65 (RelA) transcription factor is constitutively activated in human gastric carcimoma tissue. Clinical Cancer Research 7: 4136-4142

105. Sclabas GM, Fujioka S, Schmidt C, Evans DB and Chiao PJ (2003) NF-kB in pancreatic cancer. International Journal of Gastrointestinal Cancer 33: 15-26

106. Seppanen M and Vihko KK (2000) Activation of transcription factor NF-kB by growth inhibitory cytokines in vulvar carcinoma cells. Immunology Letters 74: 103-109

107. Shaffer AL, Rosenwald A and Staudt LM (2002) Lymphoid malignancies: the dark side of B-cell differentiation. Nature Review Immunology 2: 920-933

108. Shimamura T, Hsu TC, Colburn NH and Bejcek BE (2002) Activation of NF-kB is required for PDGF-B chain to transform NIH3T3 cells. Experimental Cell Research 274: 157-167

109. Soos J, Engelhardt JI, Siklos L, Hayas L and Majtenyi K (2004) The expression of PARP, NF-kB and parvalbumin is increased in Parkinson disease. Neuroreport 15 1715-1718

110. Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM and Sonenshein GE (1997) Aberrant nuclear factor-kB/Rel expression and the pathogenesis of breast cancer. Journal of Clinical Investigation 100: 2952-2960

111. Staudt LM (2000) The molecular and cellular origins of Hodgkin's disease. Journal of Experimental Medicine 191: 207-212

112. Stoffel A, Chaurushiya M, Singh B and Levine AJ (2004) Activation of NF-kappaB and inhibition of p53-mediated apoptosis by API2/mucosa-associated lymphoid tissue 1 fusions promote oncogenesis. Proceedings of the National Academy of Sciences USA 101: 9079-9084

113. Sutter AP, Zeitz M and Scherubl H (2004) Recent results in understanding molecular pathways in the medical treatment of esophageal and gastric cancer. Onkologie 27: 17-21

114. Tai DI, Tsai SL, Chang YH, Huang SN, Chen TC, Chang KS and Liaw YF(2000) Constitutive activation of nuclear factor kB in hepatocellular carcinoma. Cancer 89: 2274-2281

115. Tegeder I, Niederberger E, Schmidt R, Kunz S, Guhring H, Ritzeler O, Michaelis M and Geisslinger G (2004) Specific Inhibition of IkB kinase reduces hyperalgesia in inflammatory and neuropathic pain models in rats. Journal of Neuroscience 24: 1637-1645

116. Torabian S and Kashani-Sabet M (2005) Biomarkers for melanoma. Current Opinions in Oncology 17: 167-171

117. Tichelaar JW, Zhang Y, LeRice JC, Lam S and Anderson MW (2004) Activation of the Akt/Nuclear Factor-kB signaling axis in developing lung neoplasia. Chest 125: 153S

118. Todaro M, Zerilli M, Triolo G, Iovino F, Patti M, Accardo-Palumbo A, Gaudio FD, Turco MC, Petrella A, Maria RD and Stassi G (2005) NF-kB protects Behcet's disease T cells against CD95-induced apoptosis up-regulating antiapoptotic proteins. Arthritis and Rheumatism 52: 2179-2191

119. Toledo-Pereyra L and Lopez-Neblina F (2002) New advances in the molecular biology of ischemia/reperfusion: MAPK pathways. Tissue Antigens 60: 552

120. Trompouki E, Hatzivassillou E, Tsichritzis T, Farmer H, Ashworth A and Mosialos G (2003) CYLD is a deubiquitinating enzyme that negatively regulates NF-kB activation by TNFR family members. Nature 424: 793-796

121. Valen G, Yan ZQ and Hansson GK (2001) Nuclear factor kB and the heart. Journal of the American College of Cardiology 38: 307-314

122. Visconti R, Cerutti J, Battista S, Fedele M, Trapasso F, Zeki K, Miano MP, de Nigris F, Casalino L, Curcio F, Santoro M and Fusco A (1997) Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NFkB p65 protein expression. Oncogene 15: 1987-1994

123. Wang W, Abbruzzese JL, Evans DB, Larry L, Cleary K and Chiao PJ (1999) The NF-kB RelA transcription factor is constiutively activated in human pancreatic adenocarcinoma cells. Clinical Cancer Research 5: 119-127

124. Weber CK and Adler G (2001) From acinar cell damage to systemic inflammatory response: current concepts in pancreatitis. Pancreatology 1: 356-362

125. Whitehead IP, Lambert QT, Glaven JA, Abe K, Rossman KL, Mahon GM, Trzaskos JM, Kay R, Campbell SL and Der CJ (1999) Dependence of Dbl and Dbs transformation on MEK and NF-kB activation. Molecular and Cellular Biology 19: 7759-7770

126. Wilson SH, Caplice NM, Simari RD, Holmes Jr DR, Carlson PJ and Lerman A (2000) Activated nuclear factor-kB is present in the coronary vasculature in experimental hypercholesterolemia. Atherosclerosis 148: 23-30

127. Xiong HQ (2004) Molecular targeting therapy for pancreatic cancer. Cancer Chemotherapy and Pharmacology 54: S69-S77

128. Wratten ML, Brendolan A, Ronco C, La Greca G and Tetta C (2001) Should we target signal pathways instead of single mediators in the treatment of sepsis? Contributions to Nephrology 132: 400-414

129. Yamamoto Y and Gaynor RB (2001) Role of the NF-kB pathway in the pathogenesis of human disease states. Current Molecular Medicine 1: 287-296

130. Yang CW, Wu MS and Pan MJ (2001) Leptospirosis renal disease. Nephrology Dialysis Transplantation 16 Suppl 5: 73-77

131. Yang J and Richmond A (2001) Constitutive IkB kinase activity correlates with nuclear factor-kB activation in human melanoma cells. Cancer Research 61: 4901-4909

132. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M and Shoelson SE (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKKb. Science 293: 1673-1677

133. Zheng L, Sinniah R and Hsu SI-H (2005) In situ glomerular expression of activated NF-kB in human lupus nephritis and other non-proliferative proteinuric glomerulopathy. Virchows Archiv Oct 5: 1-12

134. Zhou X, Li F, Kong L, Tomita H, Li C and Cao W (2005) Involvement of inflammation, degradation and apoptosis in a mouse model of glaucoma. Journal of Biological Chemistry 280: 31240-31248

135. Zhu J, Hu G and Sun Y (2004) Expression and significance of nuclear factor kB in laryngeal carcinoma [Article in Chinese]. Lin Chuang Er Bi Yan Hou Ke Za Zhi 18: 745-6, 766